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13. ABSTRACT *(Maximum 200 words)*

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ASSOCIATED TO THE MICELLAR PHASE WAS FOUND TO BE a function of surfactant type, surfactant concentration, and biomass concentration. In the presence of soil, the sorption loss of surfactant onto soil were found to be significant, and capable of undermining the enhancement of the bioavailability, since the surfactant sorbed to soil also enhances the sorption of phenanthrene onto soils.

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SURFACTANT-ENHANCED INSITU BIODEGRADATION OF STRONGLY SORBING ORGANIC SUBSTANCES IN SOIL ENVIRONMENTS

Grant # AFOSR-91-0435

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Contents

This report consists of three sections:

Section A examines the biodegradation of phenanthrene when the concentration of phenanthrene in solution is below its solubility limit, and in the presence of surfactants above the critical micelle concentration. The corresponding author for this section is Dr. Peter Jaffé at Princeton University.

Section B examines the solubilization and biodegradation of excess octadecane in the presence of nonionic surfactants. This section is a summary of attachment B. The corresponding author for this section is Dr. Walter Maier at the University of Minnesota.

Section C examines the biodegradation of excess phenanthrene in soils in the presence of surfactants. This section is a summary of attachment C. The corresponding author for this section is Dr. Walter Maier at the University of Minnesota.

Attachments B and C are two thesis available from the University of Minnesota.

A "Solubilization and Biodegradation of Octadecane in the Presence of Nonionic Surfactants" by Le Thai, 1993.

B "Biodegradation of Phenanthrene in Soils in the Presence of Surfactants" by Kauser Jahan, 1993.

Table of Contents

Section A

Biodegradation of Phenanthrene in the Presence of Surfactants Above CMC

Abstract

Low-solubility Polycyclic Aromatic Hydrocarbons such as phenanthrene are easily biodegradable but due to sorption onto soil or/or their presence in a non-aqueous phase, their bioavailability is greatly reduced. In an aqueous environment where surfactants exist above their critical micelle concentration hydrophobic contaminant will partitioning into the hydrophobic core of the micelle. This enhances the apparent solubility of these hydrocarbons and therefore also their desorption from soils. Conceivably, in the absence of any inhibitory effects, such surfactants may enhance the biodegradation of the hydrocarbon. Through a set of screening experiments a group of non-ionic surfactants were identified which do not inhibit the bacterial degradation of the phenanthrene. A mathematical model was formulated to describe the interaction of the biomass-contaminant-water-surfactant system. The model assumes that the surfactant affects the solubility of phenanthrene and does not affect the biochemical characteristics of the biomass. An effective bioavailable concentration was defined. The model predicts experimental data well indicating that, a part of the phenanthrene in the micelle phase can be degraded simultaneously with the phenanthrene in the aqueous phase. The bioavailable fraction of phenanthrene associated to the micellar phase was found to be a function of surfactant type, surfactant concentration, and biomass concentration. In the presence of soil, the sorption loss of surfactant onto soil were found to be significant, and capable of undermining the enhancement of the bioavailability, since the surfactant sorbed to soil also enhances the sorption of phenanthrene onto soils.

Introduction

Polycyclic Aromatic Hydrocarbons (PAH) are pollutants of major concern in the remediation process of oil spill sites. Low volatility, coupled with hydrophobic characteristics, make them more persistent in nature. Biological transformation is believed to be the principal process, affecting the removal of PAH contaminants. The hydrophobic nature of these contaminants results in their partition onto the soil matrix. In most cases this can account for 95% to 99% of the total contaminant mass. This limits the biological transformation by reducing the soluble concentration, thereby, making them unavailable to the microbial population. A well designed bioremediation process should consider methods to mobilize these contaminants from the soil surface, to make them available to the microbial population. At the same time, additives that enhance the desorption should not have a great oxygen demand, so that sub-surface oxygen and other nutrients become limiting.

Surfactants have been found to be effective in solubilizing hydrophobic contaminants from soil surface [Ellis et al. (1985)]. Surfactant molecules above their Critical Micelle Concentration (CMC), form aggregates in water which are called micelles. These aggregates have a hydrophobic core and a hydrophilic outer surface. Micelles are capable of dissolving hydrophobic PAH compounds at its hydrophobic core resulting in an increased apparent aqueous solubility of the compound. Solubilization depends on the type and dose of the surfactant, the hydrophobicity of the contaminant, the surfactant-soil interaction, and the time that the contaminant has been in contact with the soil [Vigon and Rubin (1989)].

Pump-and-treat aquifer remediation schemes are often hindered by the low solubility of the hydrophobic compounds. Over the last few years, attention has been focused on increasing the solubility of these contaminants with the addition of surfactants, leading to some studies involving laboratory and field experiments of above-ground and in-situ soil washing [Clarke et al. (1991]. Some model studies have been conducted to predict and design a surfactant-enhanced remediation scheme [Edwards (1991); Megehee et al. (1993)]. Significant solubilization occurs above the CMC of the surfactant, and most of the very low-solubility contaminants are solubilized in the surfactant micelles. Disposing of this micelle-contaminant spent wash is often a major problem. This can be made efficient if the contaminant bound to micelle can be biodegraded in above-ground reactors or in-situ. The focus of this project is to study the degradability of the micelle phase contaminant.

Surfactants are known for their capability in enhancing biodegradation of oil spills in the open water, by reducing the surface tension and the droplet size [National Research Council, (1989)]. A few field experiments in the past indicated potential for enhanced biodegradation of sub-surface PAH contaminants in presence of surfactant s [Rittmann and Johnson (1989)]. A few laboratory studies indicate an enhanced rate of degradation of PAH contaminants in presence of some non-ionic surfactant below CMC [Aronstein et al. (1991); Aronstein and Alexander (1993)]. Whereas other investigators [Laha and Luthy (1991)], observed strong inhibition of the biodegradation of Phenanthrene in the presence of some nonionic surfactants above their CMC.

However, the available literature lacks a systemic study towards understanding the effect of surfactants on the bioavailability of hydrophobic pollutants associated with a micellar phase, limiting their result to specific surfactants, surfactant doses, and contaminants. The objective of this part of this project is to understand the mechanism of the biodegradation of a hydrophobic contaminant associated to the micellar phase. In this project, we will addressed the following:

a) Explain the mutually contradicting result reported in the literature, cited above, and suggest simple screening experiments for choosing surfactants that do not inhibit the biodegradation of hydrophobic organics.

b) With the help of experiments and mathematical simulations we will investigate the biodegradability of the contaminant associated with the micellar phase, and suggest a kinetic formulation for the degradation of the same.

c) Assess batch experiments and methods for their interpretation to determine the Monod kinetic coefficients for low-solubility, volatile PAH.

Experimental Methods and Materials

The work conducted consisted of a series of batch experiments. Kinetics of the degradation of phenanthrene were monitored as a function of the surfactant concentration in the presence and absence of soil. Listed below are the procedures and materials that we were use to conduct these experiments.

Experimental Procedures

Determination of CMC

A concentrated surfactant solution was prepared by dissolving 1 ml of surfactant (1g for solid surfactants) in 50 ml deionized water. Appropriate dilutions were prepared and their surface tensions measured with a surface tensiometer (Fisher Scientific Surface Tensiomat 21). Surface tensions were plotted against the logarithm of the surfactant concentration. The surfactant concentration at which the surface tension no longer decreases significantly is the CMC.

Solubility Enhancement Experiments

A series of 15 ml centrifuged tubes were spiked with a mixture of ^{14}C-phenanthrene and non-labeled phenanthrene, dissolved in methanol. The mass of phenanthrene added was in excess (about two times) of the solubility limit. Methanol was then allowed to evaporate, after which surfactant solutions of varied concentration were added to each tube. Tubes were placed in a rotary shaker for 7 days, at 20 ^{o}C. At the end of the incubation period, samples were taken in duplicate, from each tube, and passed through 0.1 μm syringe filters, to eliminate any granular phenanthrene. Filters were pre-saturated with phenanthrene to minimize the adsorption from the samples. Samples were then analyzed for radioactivity in a Liquid Scintillation Counter, in order to quantify the phenanthrene concentration in solution.

Sorption of phenanthrene to soil

A stock solution of radiolabeled phenanthrene with an initial concentration near its solubility limit and a known initial radioactivity was prepared. A series of 15 ml centrifuge tubes were set up with varying amounts of soil in them. Ten ml of stock solution were pipetted into each of the centrifuge tubes. After capping the tubes, they were placed into a rotary shaker at 20 ^{o}C for 48 hours. At the end of the incubation time the tubes were centrifuged at ~600 x g for 30 minutes. The supernatant of each tube was analyzed for radioactivity, in order to quantify the concentration of phenanthrene in solution and determine he sorbed phenanthrene by difference.

Sorption of surfactant to soil

These experiments were performed similarly to those described above. The surfactant concentration was analyzed by diluting it below its CMC and measuring the surface tension.

Sorption of phenanthrene onto the biomass

These experiments are similar to those described for soil with the exception that a known amount of biomass was added to each tube in lieu of soil. Incubation time was fixed at 30 minutes to avoid any significant biodegradation during this time period.

Sorption onto the Apparatus

These experiments were required for a proper correction of the overall mass of phenanthrene/surfactant in the system. Fifteen ml of phenanthrene/surfactant solution of varying concentrations were added to each flask and stirred for 5 minutes. Concentration of phenanthrene/surfactant were then analyzed.

Biodegradation Experiments

These experiments were conducted in 25 ml reaction vessels, that contain in their headspace a cup in which the CO_2 that evolves during the biodegradation can be absorbed into a KOH solution (figure 1). Stoppers were lined with aluminum foil to minimize losses due to sorption from the headspace. A solution (15 ml) containing phenanthrene, seed, surfactant, and soil (or any combination as appropriate for a particular experiment) were placed in a reaction vessel. A volume of 0.25 ml of 1N KOH was placed in the KOH cup. Reaction vessels were sealed and placed on a magnetic stirrer to mix the reactant continuously. At the end of the desired reaction time, concentrated sulfuric acid was introduced into the vessels to stop the reaction and to release any dissolved carbon dioxide from the aqueous solution. After an additional 6-8 hour period, samples from the KOH cup and reaction chamber were analyzed for radioactivity, to determine the mass of carbon dioxide produced and the loss of phenanthrene respectively.

The biodegradation of the surfactant and the effect of the surfactant on the biodegradation of non the hydrophobic substrate, were studied in a HACH BOD Manometric apparatus, model 2173B.

Analytical Procedures

^{14}C labeled Phenanthrene was analyzed with a Packard 1900 TR Liquid Scintillation Counter using Scintiverse BD scintillation cocktail from Fisher Scientific. A linear transformation converts the disintegration per minute data to phenanthrene concentration.

Biomass was measured as Total Organic Carbon, in a Coulometrics TOC-TC analyzer. Liquid samples were oxidized at 900°C in a pure oxygen environment in the presence of a barium chromate combustion catalyst/scrubber. The carbon dioxide produced was measured in a CO_2 Coulometer (ASTM D4129 - 82).

Surface Tension of the air/water interface was measured using a Fisher Surface Tensiomat, model 21. This instrument measures the force necessary to pull a platinum-iridium ring free from the interface.

B O D was measured using HACH manometric apparatus, model 2173B. Appropriate amounts of sample and seed were placed in the bottle which was connected to a closed end manometer, thus forming a closed system. The carbon dioxide produced is removed by placing lithium hydroxide crystals in the rubber cup near the cap. Oxygen consumption is proportional to the pressure drop in the system which is indicated in a pre-calibrated scale.

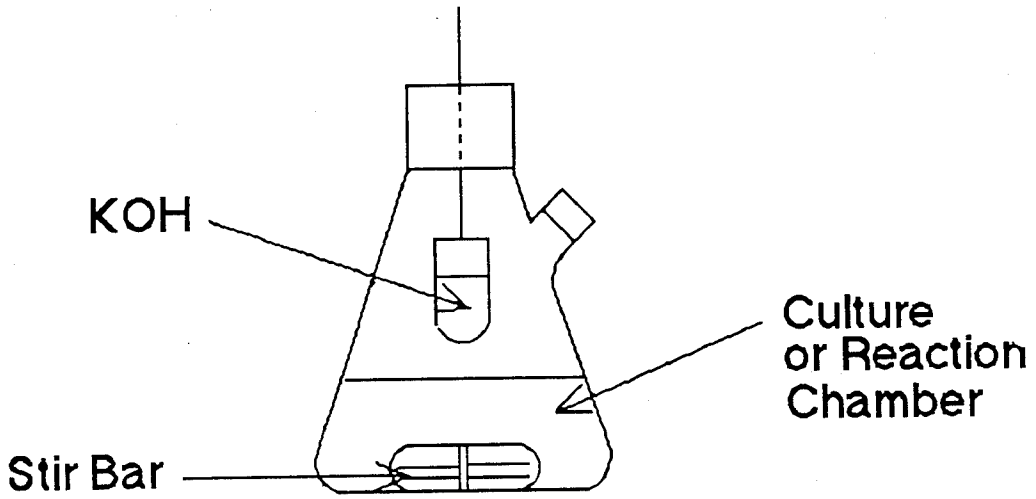
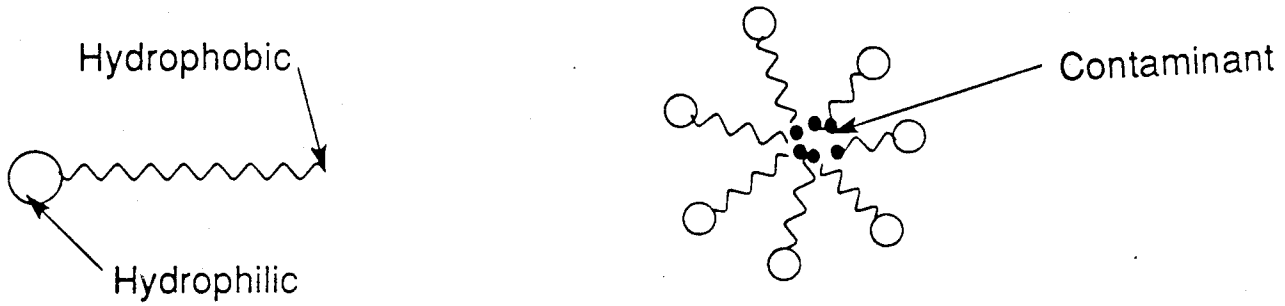


Figure 1. Experimental Setup



a. Non-ionic Surfactant Monomer

b. Contaminant Partitioned in Micelle

Figure 2

Materials

Contaminant

Phenanthrene was used as a representative PAH compound. It is a hydrophobic compound with very low aqueous solubility [1.29 +/- 0.07 mg/l at 25 ^{o}C, Stephen and Stephen (1963)]. Although volatile in the aqueous solution, very little volatilization loss (< 0.01%) was reported [Park et al. (1990)] from soil sample after 48 hours of incubation. Phenanthrene is easily biodegraded in the presence of an appropriate microbial population.

^{14}C-labeled phenanthrene was mixed with non-labeled phenanthrene to obtain a desired radioactivity in the solution.

Surfactant

Several commercially available surfactants were screened. All of them are water soluble, bio-degradable, and capable of increasing the apparent solubility of hydrophobic organic compounds. Some are pure compounds while others are a mixture of different surfactants. These surfactants along with their chemical formula are listed in Table 1. The surfactants were selected based on the following criteria:

a) Water Soluble;

b) Capable of solubilizing hydrophobic compounds;

c) Non-hazardous, and biodegradable in the presence of an appropriate culture.

Soil

The soil used was a local surface soil/sediment, that we have characterized previously in terms of its organic carbon content (Table 2), and sorption of non-ionic organic pollutants as well as non-ionic and anionic surfactants.

Biomass

A mixed culture, (identical to the one used by Prof. Walter Maier from the Univ. of Minnesota) was maintained in the laboratory. This culture is capable of using phenanthrene as a sole carbon source. An active enrichment culture was maintained by wasting two thirds of the volume of the culture twice a week, and replacing it with a fresh nutrient solution and phenanthrene.

BOD dilution water was used as nutrient solution. Two types of cultures were maintained: In one, 0.025 g of solid phase phenanthrene were added to a 100 ml batch reactor. In the second system, a 5 ml solution of 0.02 g/ml of phenanthrene in methanol were injected with a syringe pump over a two day period into the reactor. Both the cultures yielded similar result for the degradation kinetics of phenanthrene.

Table 1. Characteristics of the Surfactants

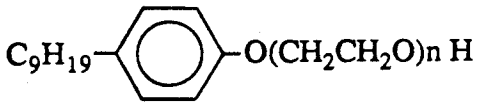
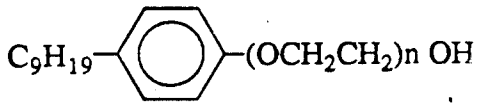
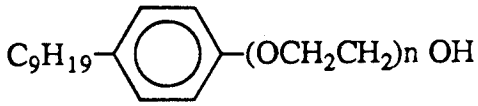
Surfactants	Formula	CMC, mg/L	CMC With 5% Soil, mg/L	Result
Triton X-102	 n=9.5	150	510	(-)ve
Triton N-101	 n=9	63	125	(+)ve
Tergitol NP-10	 n=10.5	52	550	(-)ve
Polyoxyethylene 10 Lauryl Ether	$C_{12}H_{25}(OCH_2CH_2)n\ OH$ n=10	48	250	(+)ve
Triton CF-21	NA	210	420	(+)ve
Tergitol 15-S-9	NA	70	460	(+)ve
Tergitol 15-S-20	NA	105	1050	(-)ve
Tergitol TMN-10	NA	1200	2500	(-)ve

Table 2. Physical Characteristics of the Soil

Sample Fraction	Size Range	Percent by Weight	Percent Organic Carbon Content
Bulk	< 2mm		2.60
Sand	2mm - 62μm	17	0.58
Silt	62μm - 2μm	25	1.85
Clay	< 2μm	58	3.51

Results and Discussion

Determination of CMC

Surfactants are classified by ionic type e.g. anionic, cationic, and nonionic surfactants. Cationic surfactants sorb strongly onto soils and clays, mainly by cation exchange and are therefore not less useful for the mobilization of contaminants. For this work we have examined only a series of nonionic and anionic surfactants.

A typical surfactant molecule (monomer) has a two component molecular structure (figure 2a). One component is hydrophilic while the other is hydrophobic. Above certain concentration, a number of monomers combine to form aggregates (micelle) of different shapes like spheroid, oblate spheroid etc. Each monomer is oriented with their hydrophilic end projected outwards and the hydrophobic ends towards the center. The concentration of surfactant at which these micelles start forming is referred to as the Critical Micelle Concentration (CMC). Micelles have therefore a hydrophobic core at their center, into which hydrophobic pollutants can partition (figure 2b).

The CMC for all of the selected surfactants was determined as described earlier. A typical result is shown in figure 3. Table. 1 shows the list of surfactants selected with their measured CMC's.

Screening Experiments

The goal of the screening experiments was to identify surfactants that would not inhibit to a very large degree the bacterial degradation of phenanthrene, when the surfactant concentration is above CMC.

A series of reaction vessels were set up for different doses of surfactant. Parallel sets were run in the presence (5%, w/w) and absence of soil. Blanks runs were also set up without surfactant. Carbon Dioxide production in each reaction vessel was measured after 15 days in order to quantify the degree of mineralization of phenanthrene. Identical experiments were conducted for each of the different surfactants.

Some of the surfactants did not show a significant effect on the degradation of phenanthrene, while others reduced the degradation dramatically. For several surfactant types and doses nearly as much carbon dioxide was produced after 15 days of incubation in the presence of the surfactant as in their absence (figures 4a to 4d). In contrast, for other surfactants (figures 5a to 5d) significantly less carbon dioxide was produced in their presence. We label the first set as (+) and the later as (-).

The above results indicate that the inhibition of the degradation of phenanthrene by surfactants above their CMC is dependent on the surfactant type. This would explain some of the contrasting results reported in the literature.

To investigate the effect that surfactants above their CMC have on the biodegradation of low-solubility hydrocarbons further, we concentrated on the surfactants labeled (+). The respective experiments and interpretations are described in the remainder of this chapter.

Figure 3: Determination of CMC for Triton N101

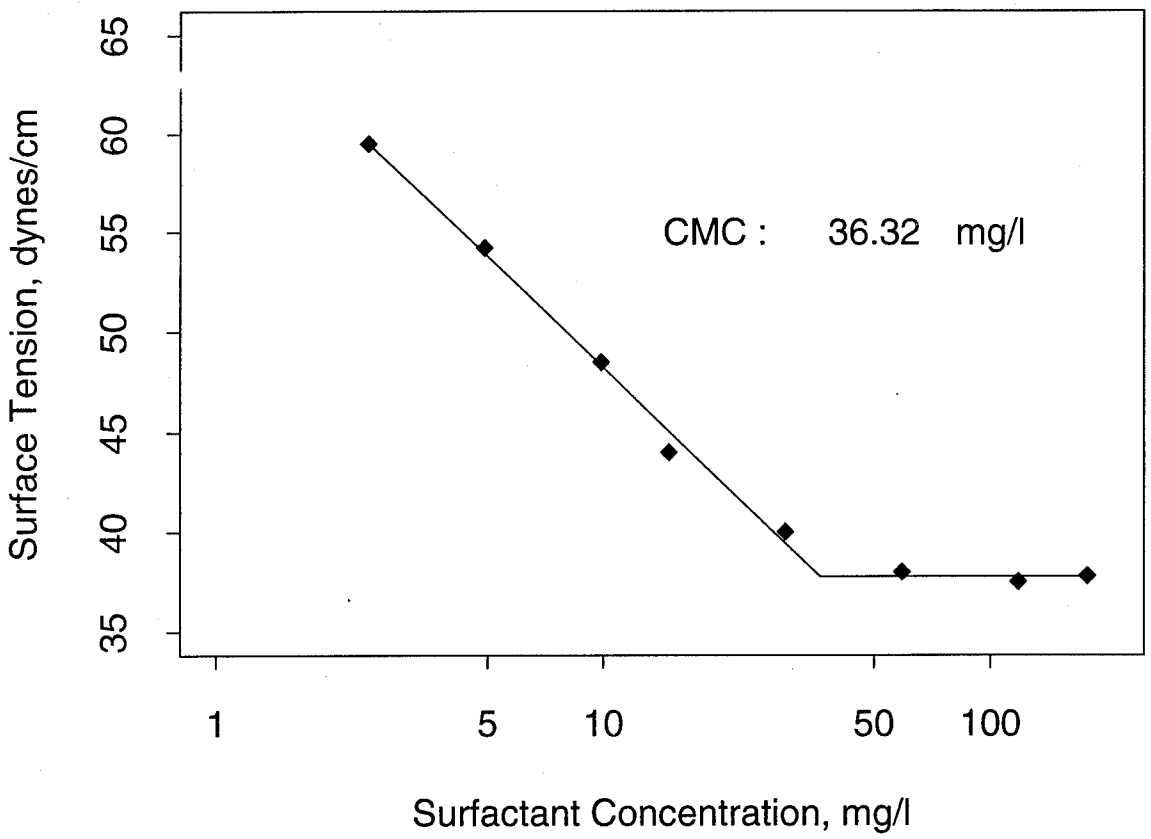


Figure 4a: Carbon Dioxide Production after 15 days with Triton N101

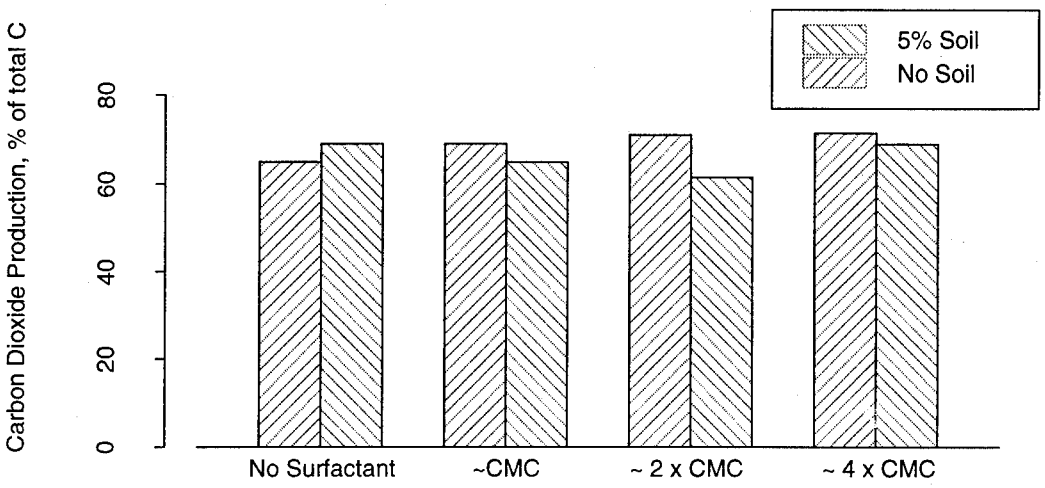


Figure 4b: Carbon Dioxide Production after 15 days with Triton CF21

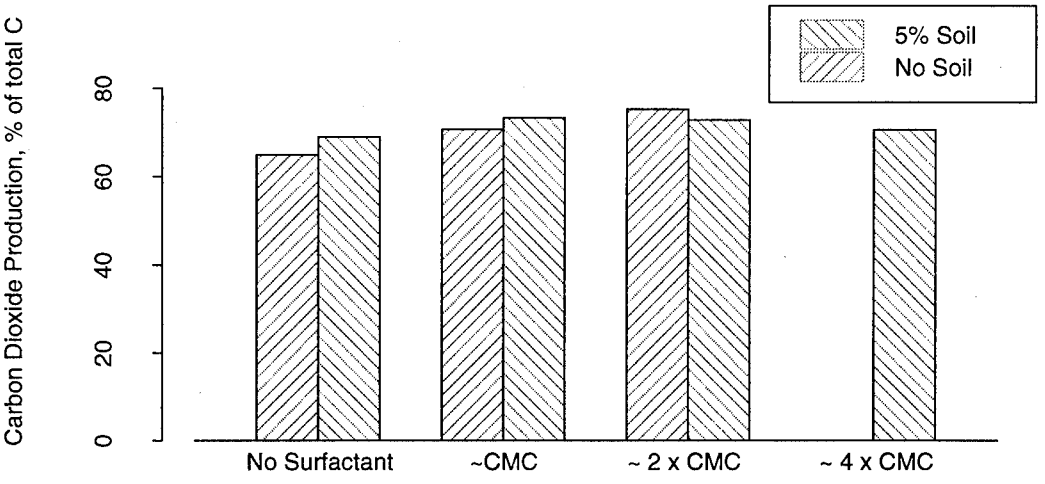


Figure 4c: Carbon Dioxide Production after 15 days with Tergitol 15-S-9

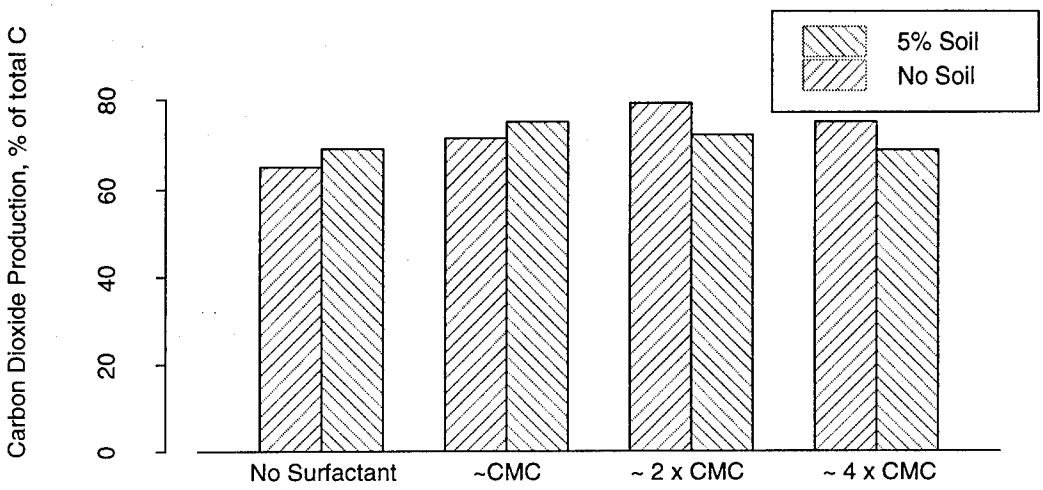


Figure 4d: Carbon Dioxide Production after 15 days with Polyoxyethylene 10 Lauryl Ether

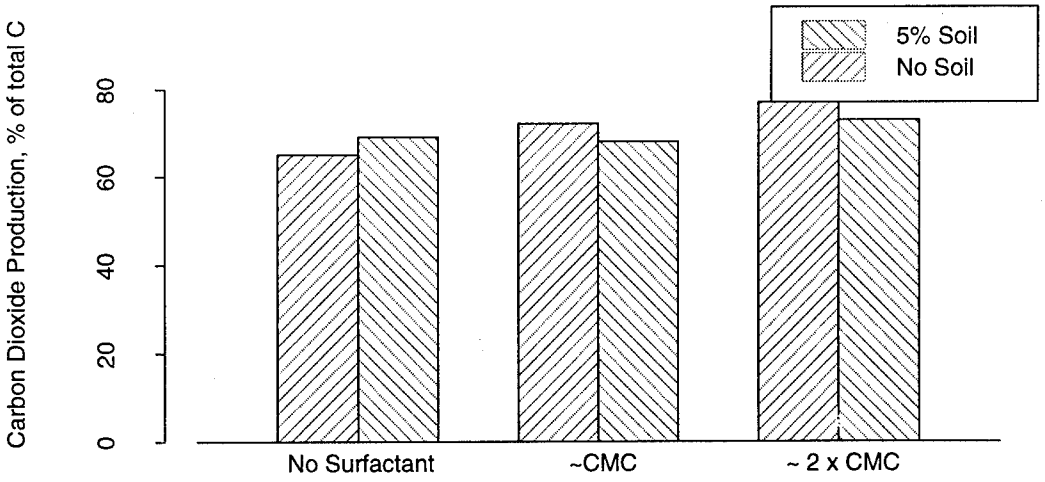


Figure 5a: Carbon Dioxide Production after 15 days with Triton X102

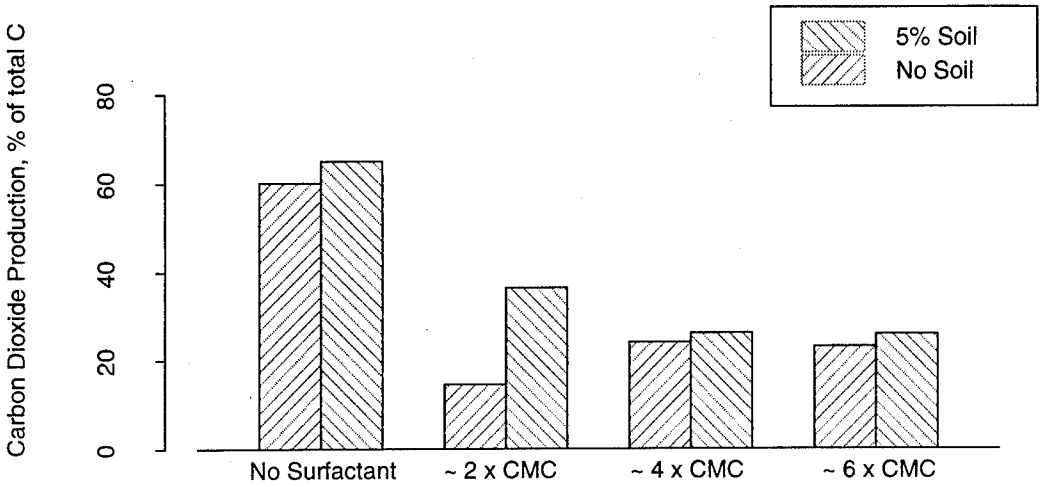


Figure 5b: Carbon Dioxide Production after 15 days with Tergitol 15-S-20

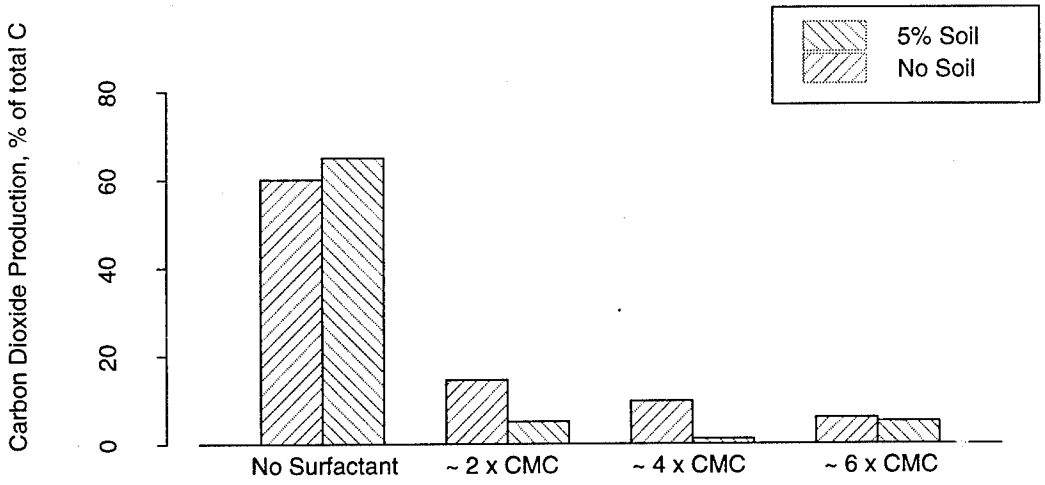


Figure 5c: Carbon Dioxide Production after 15 days with Tergitol NP10

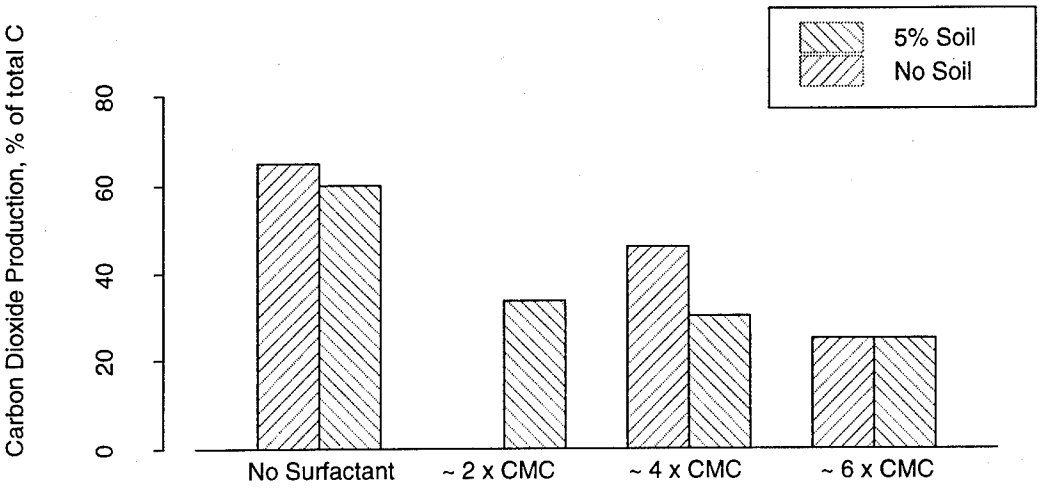
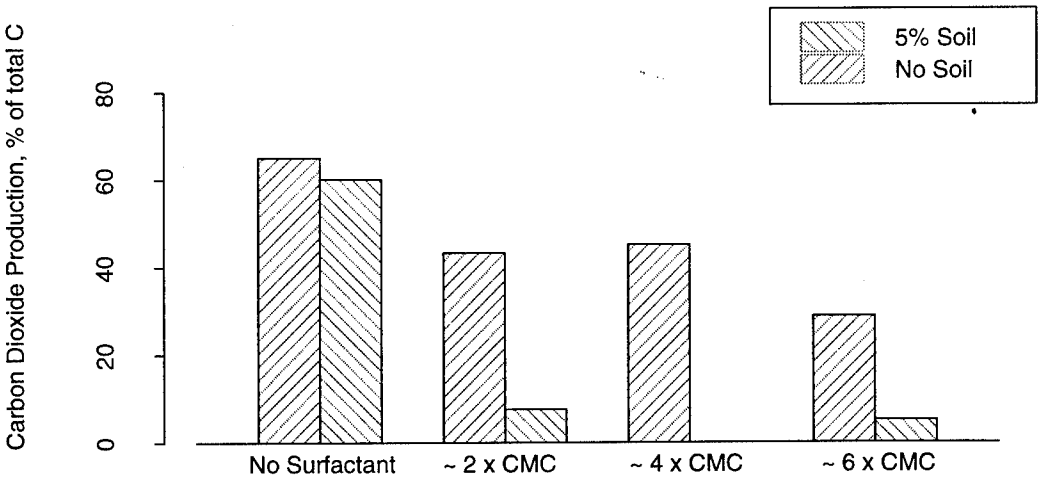


Figure 5d: Carbon Dioxide Production after 15 days with Tergitol TMN10



Surfactant Solubilization

The solubilization of phenanthrene by the surfactant can formulated as follows:

$$C_{mon} = X_{mn} K_{mn} C$$
$$C_{mic} = X_{mc} K_{mc} C$$

where X_{mn} and X_{mc} are given by:

$$X_{mc} = \begin{cases} X - CMC.for.X > CMC \\ 0.for.X < CMC \end{cases}$$
$$X_{mn} = \begin{cases} X.for.X < CMC \\ CMC.for.X > CMC \end{cases}$$

where,

C_{mon} = phenanthrene bound to monomers, mg/l;

C_{mic} = phenanthrene bound to micelles, mg/l;

C = phenanthrene dissolved in the water, mg/l;

X = total surfactant concentration, mg/l;

X_{mn} = surfactant in monomer form, mg/l;

X_{mc} = surfactant in micelle form, mg/l;

K_{mn} and K_{mc} = partition coefficients.

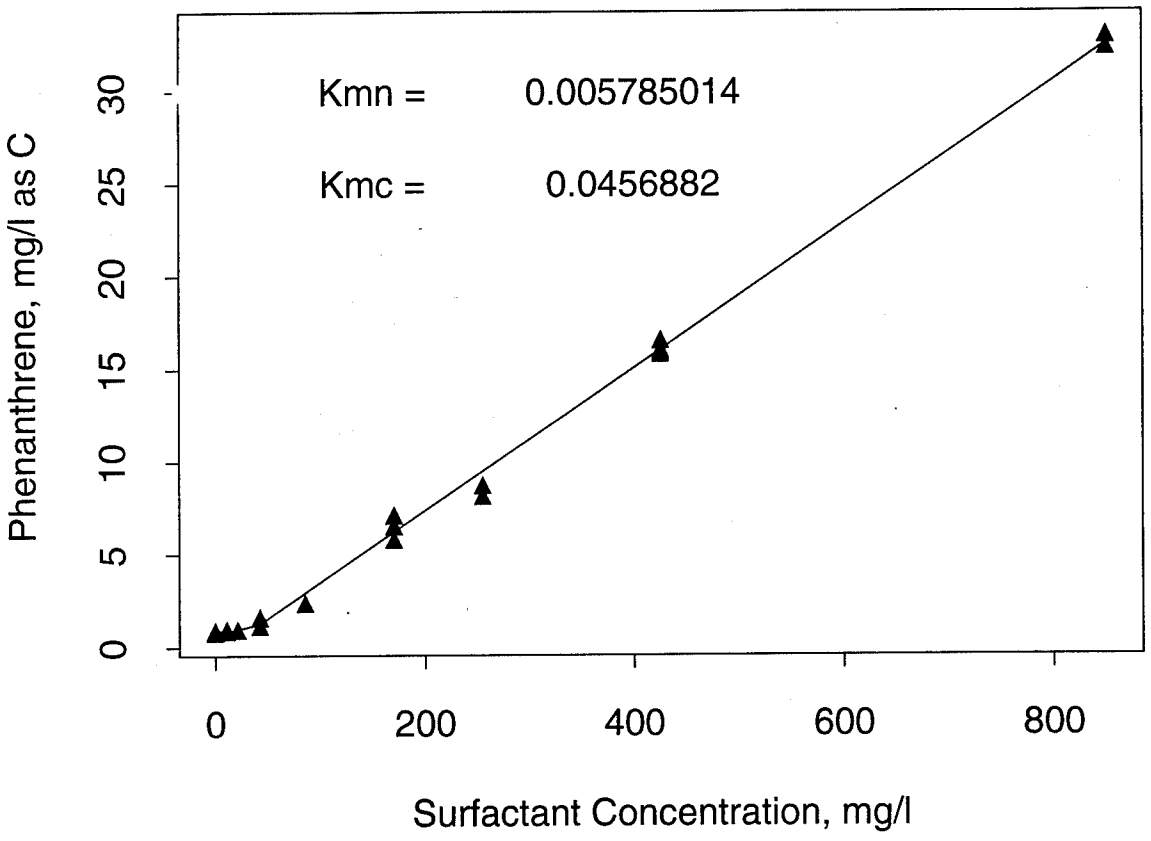
The enhanced solubility of phenanthrene in presence of Triton N101 is shown in figure 6. A sharp change in slope can be seen at the CMC. Values of K_{mn} and K_{mc} are about an order of magnitude different, as can be seen from the results shown in figure 6. The total apparent soluble concentration of phenanthrene is equal to $(C + C_{mon} + C_{mic})$.

Sorption of Phenanthrene onto Soil

The sorption of phenanthrene onto soil can be described at equilibrium as:

$$C_s = K_d C$$

Figure 6: Enhanced Sollubility of Phenanthrene by Triton N101



where,

C_S= sorbed concentration of phenanthrene on soil (mg/g),

C= dissolved concentration of phenanthrene (mg/l),

K_d= partition coefficient (l/g).

The sorption isotherm of phenanthrene for the soil is shown in figure 7 with the value of K_d indicated therein. The value of the partition coefficient normalized with respect to the organic carbon content of the soil was found to be 1.494×10^{-2} l/mg, which compares well with measurements reported in the literature. For a 5% soil dose (as used in our experiments), nearly 95% of the total phenanthrene mass is sorbed onto the soil.

Sorption onto the Biomass

Biomass is capable of sorbing a sizable portion of hydrophobic contaminants like phenanthrene. At equilibrium, this sorption can be described as:

$$C_{bms} = k_{oc} C$$

where,

C_{bms} = phenanthrene sorbed to biomass, mg/mg;

C = phenanthrene dissolved in water, mg/l;

k_{oc} = partition coefficient, l/mg.

Biomass was expressed as Total Organic Carbon (TOC). The sorption isotherm of phenanthrene onto the biomass is shown in figure 8. The value of k_{oc} (which for this case is equal to the partition coefficient normalized with respect to the organic carbon content) was found to be 1.39×10^{-2} l/mg, which is similar to that onto the soil-organic carbon given above.

Sorption onto the Apparatus

The glass reactor vessels were found to sorb some amount of phenanthrene which can also be described by a linear partition equilibrium isotherm:

$$C_g = K_{dg} C$$

where,

C_g = phenanthrene concentration on the glass (expressed in equivalent phenanthrene concentration insulation), mg/l;

Figure 7: Sorption Isotherm of Phenanthrene on Soil

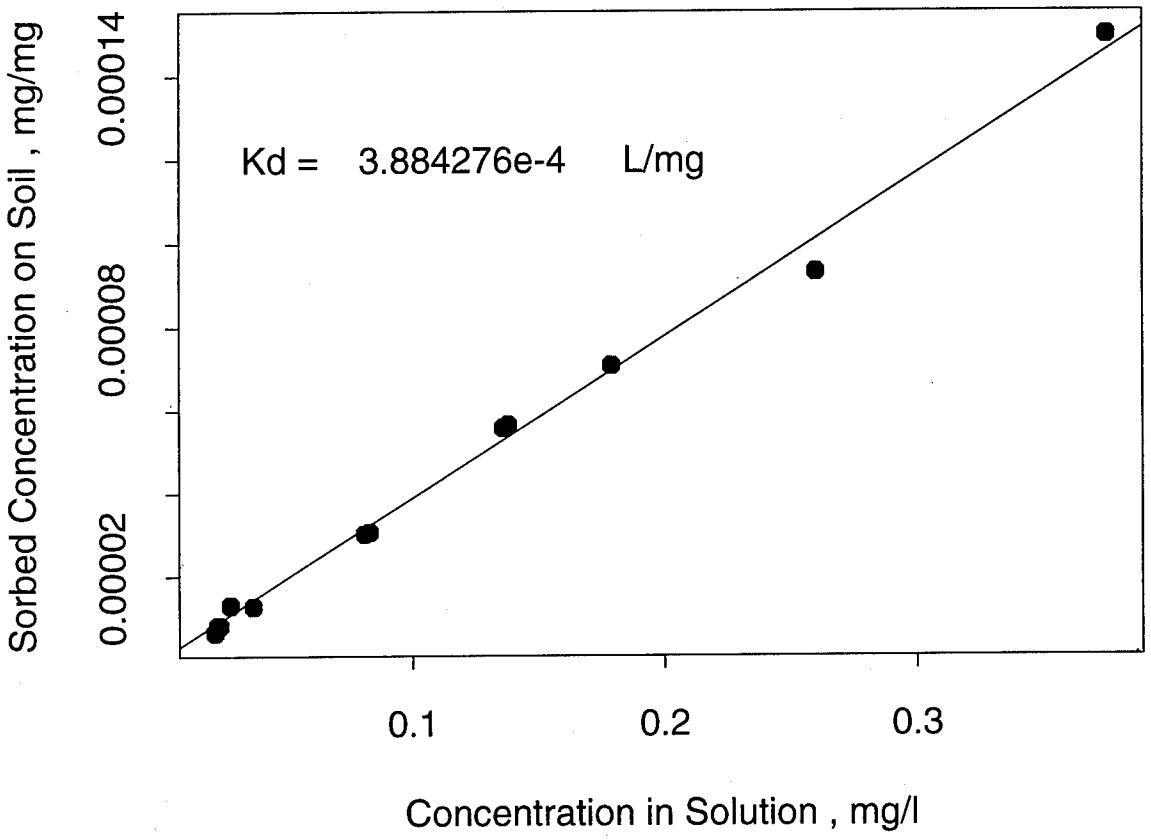
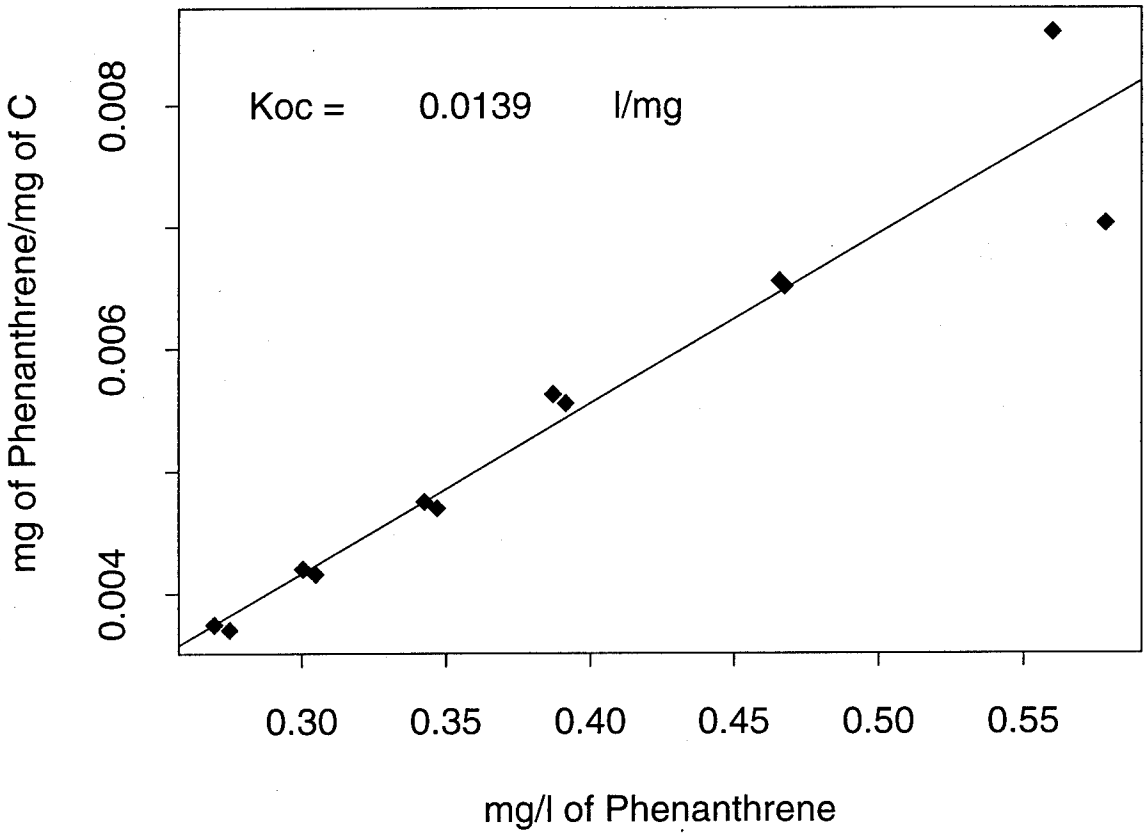


Figure 8: Sorption Isotherm of Phenanthrene on Biomass



C = phenanthrene concentration dissolved in water, mg/l;

K_{dg} = partition coefficient, dimensionless.

By keeping the volume of reactants constant for all experiments we obtained a dimensionless and constant partition coefficient (K_{dg}) for our experimental set-up, which in this case equals 0.1596 (figure 9).

Biological Parameters, μ_m and K_S

Biodegradation experiments were performed without the addition of either surfactants and soil. Frequent sampling was conducted to determine the carbon dioxide (CO_2) and phenanthrene concentration (C) profiles during the growth-phase and beyond. An optimization problem was formulated with the following assumptions:

a) Residuals in the values of C and CO_2 are normally distributed.

b) Residuals in individual experiments are independent.

c) All the experiments have the same co-variance matrix for the distribution of residuals.

Based on these assumptions, a Likelihood equation can be stated as:

$$\text{Minimize, } f(\theta) = \frac{n}{2}\log[\det.M(\theta)] \quad \text{subject to, } \theta = \begin{pmatrix}\theta_1\\ \theta_2\end{pmatrix} = \begin{pmatrix}\mu_m\\ K_s\end{pmatrix} \geq 0$$

where,

n = number of observation;

M(q) = Moment matrix of residuals given by,

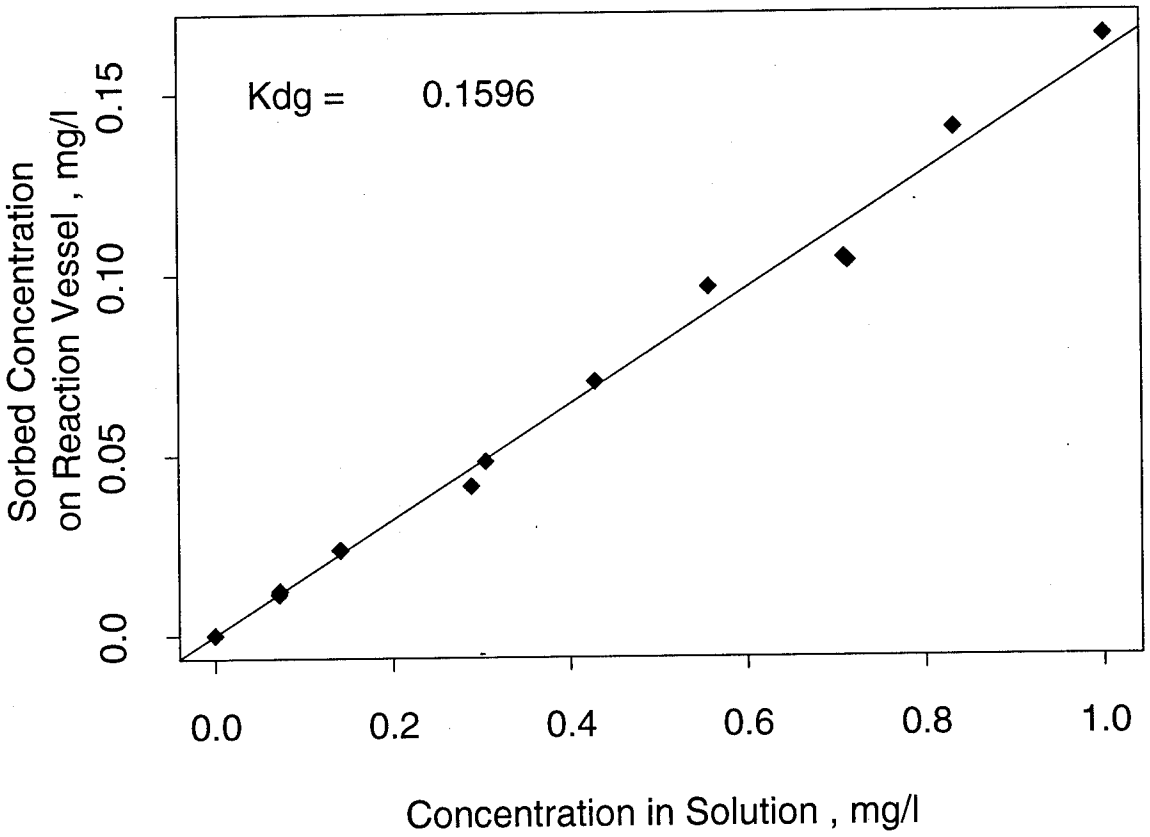
$$M(\theta) = \sum_{\mu=1}^{n} e_\mu(\theta) e_\mu^T(\theta) = \begin{bmatrix} \sum e_c^2 & \sum e_c e_{co_2} \\ \sum e_c e_{co_2} & \sum e_{co_2}^2 \end{bmatrix}$$

where, $e_c = \hat{c} - c$ and $e_{co_2} = \hat{co}_2 - co_2$ are the residuals, the difference between the observed and computed values of C and CO_2, obtained from the following set of non linear differential equations:

Contaminant (C) Balance:

$$\left(1 + K_{dg} + k_{oc} S\right)\frac{dC}{dt} = -\frac{\mu_m}{Y}\frac{C}{K_s + C}S$$

Figure 9: Sorption Isotherm of Phenanthrene on Experimental Setup



Biomass (S) Balance :

$$\frac{dS}{dt} = \mu_m \frac{C}{K_s + C} S - bS$$

Carbon Dioxide (CO_2) Production :

$$\frac{d[CO_2]}{dt} = \mu_m \left(\frac{1-Y}{Y}\right) \frac{C}{K_s + C} S + \text{Contribution from Endogenous Respiration}$$

with the initial conditions:

$C = C_o$, $S = S_o$, and $CO_2 = 0$ at $t = 0$.

b is Endogenous decay coefficient which was determined by a separate set of experiment analyzing a decaying biomass population. Result and value is shown in figure 10.

The Yield coefficient (Y) was computed from the data reported by Krauser (1993) as 0.35 mg/mg (as carbon).

The above optimization problem was solved using the Davidon-Fletcher-Powel non-linear optimization algorithm, a variable metric method. Cubic interpolation was used for calculating optimum step sizes. Euler backward time stepping with Picard iteration scheme was used for the solution of the set of coupled nonlinear initial value problem. The value of μ_m and K_S were found to be (0.55 +/- 0.1) x 10^{-2} /hour and (4.4+/-1.0) mg/l respectively.

A plot of the optimum surface is shown in figure 11. It can be seen that the optimum solutions (along the μ_m vs. K_S surface) occur along a valley that extends like a narrow semi-infinite elliptic region defining pairs of μ_m and K_S values that give almost equally good fits. The pair with the minimum K_S value was chosen for our purpose, given the low limit of solubility (~1.29 mg/l) of phenanthrene.

The distribution of C and CO_2 residuals is shown in figures 12a to 12d. The Quantile-Quantile plot with standard normal is linear for CO_2 (figure 12c), whereas the residuals on C (figure 12a) shows a few suspect outliers. The histogram for the CO_2 residuals shows a near normal distribution centered at origin (figure 12d). The histogram for residuals on C (figure 12b) is not centered at zero, indicating some bias. This bias was thought to have been caused by a systematic loss in the experimental setup.

Biodegradation in the Presence of Surfactant

So far we have not considered the biodegradation of the phenanthrene in presence of surfactant solutions. We have defined the partition processes of phenanthrene onto soil, biomass, micellar phase, and glassware, and have determined the appropriate equilibrium sorption/partition parameters. We have also determined the biological degradation, growth and decay parameters for phenanthrene. We will now formulate a model from the physical process

Figure 10: Endogenous Respiration Coefficient for the Mixed Culture

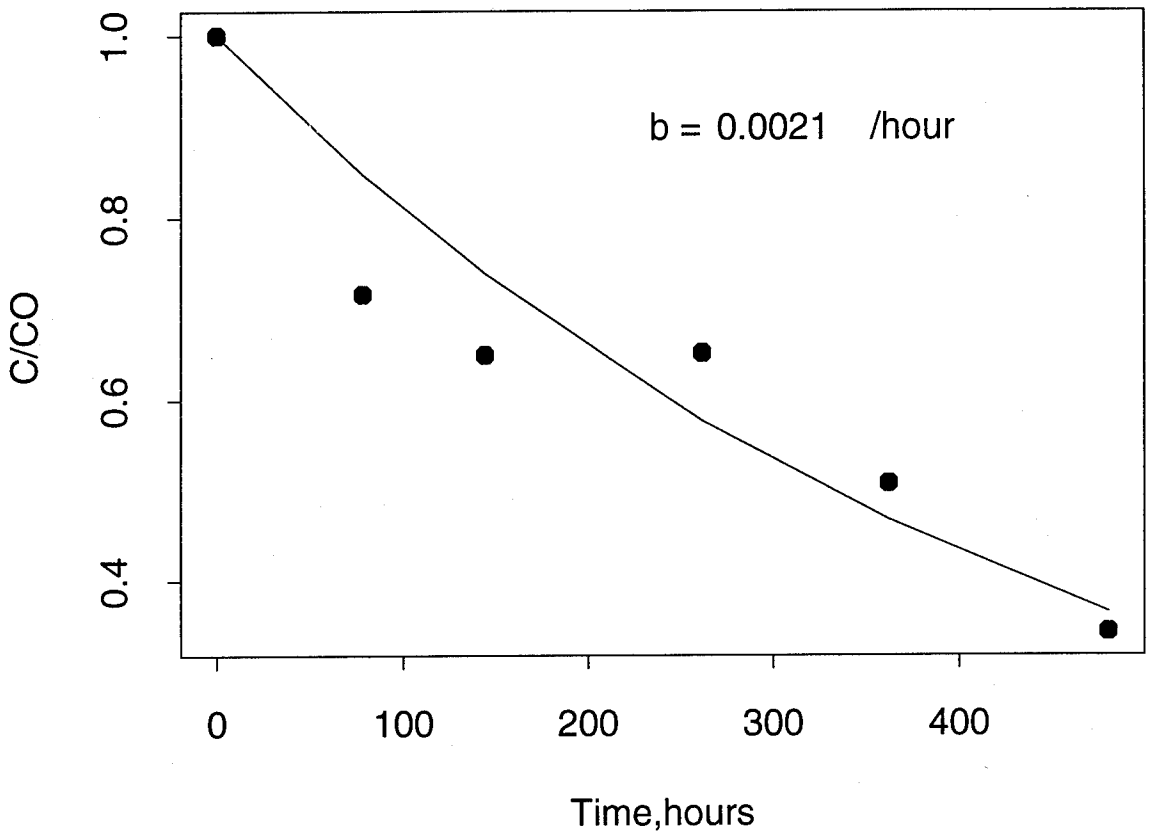


Figure 11: Optimum Surface of the Likelihood Funtion for Um and Ks

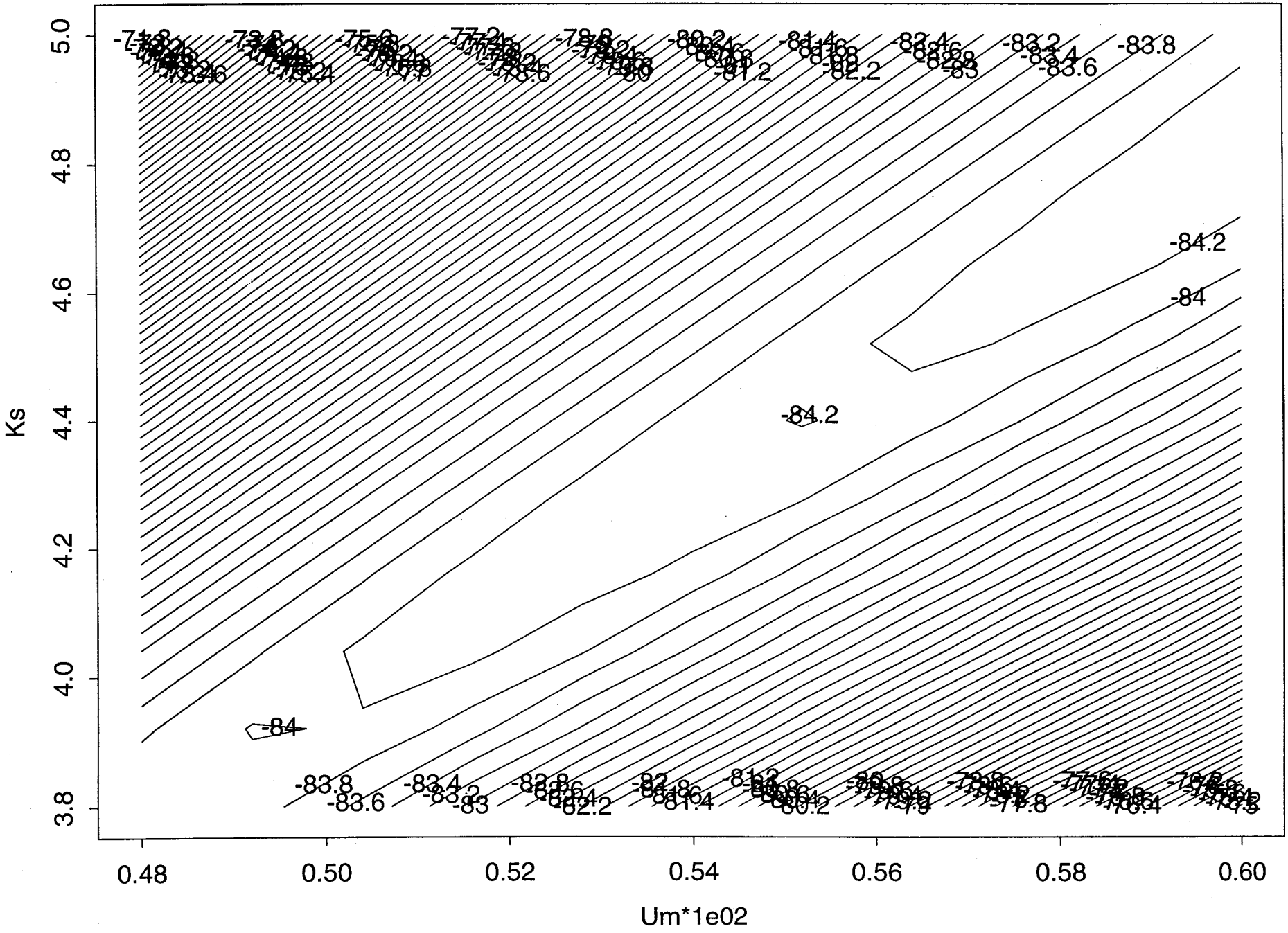


Figure 12a: Q-Q Plot with Normal for Residuals on Phenanthrene Concentat

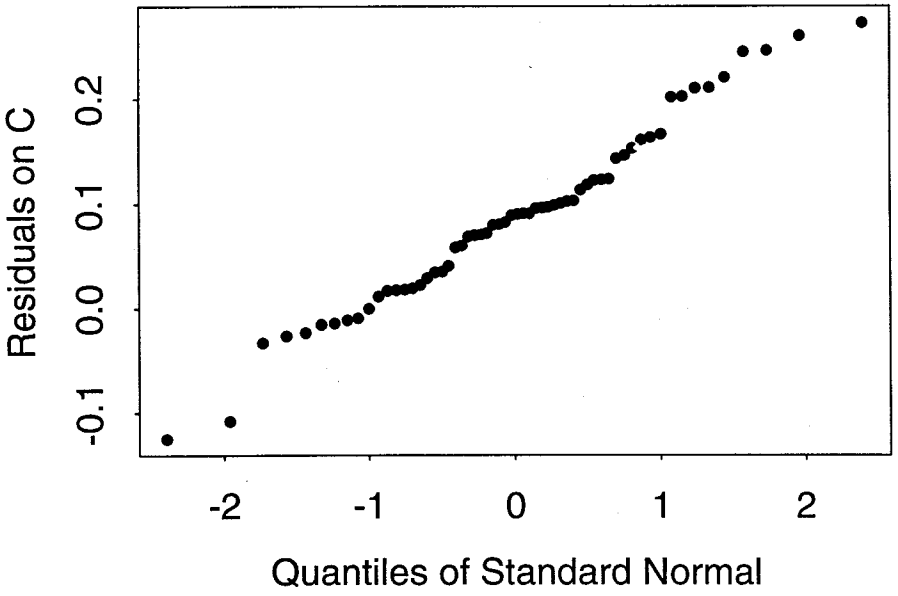


Figure 12b: Histogram of Residuals on Phenanthrene Concentation

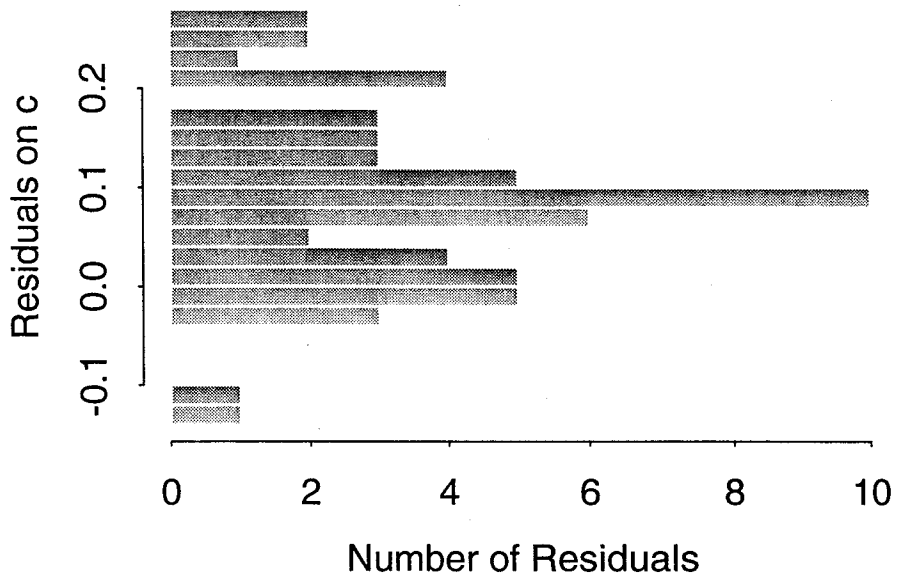


Figure 12c: Q-Q Plot with Normal for Residuals on Carbon Dioxide

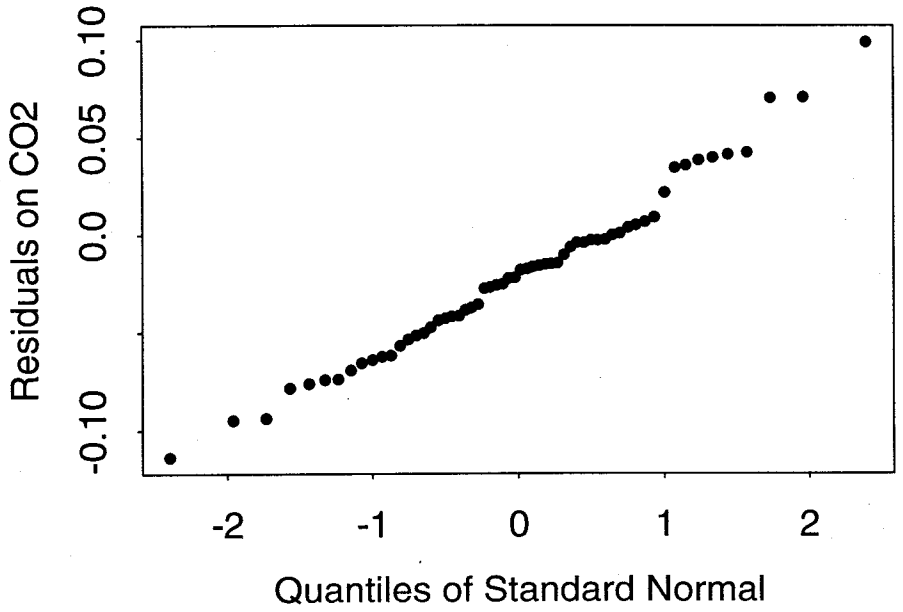
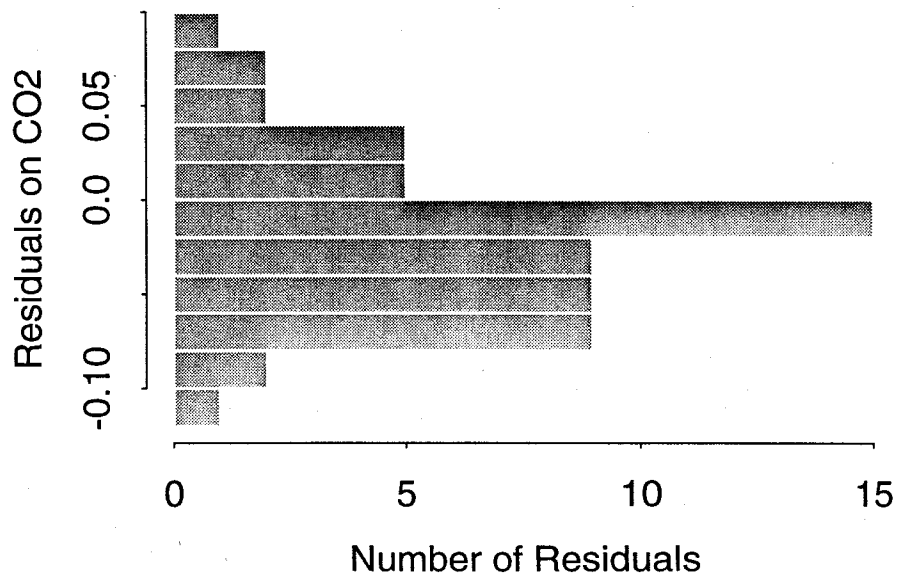


Figure 12d: Histogram of Residuals on Carbon Dioxide



considerations, for the degradation of phenanthrene in presence of a surfactant solution above CMC. In formulating the model we will make the following assumptions:

a) Transport is not rate limiting (i.e. the system is completely mixed).

b) Transfer of contaminant between different phases is instantaneous relative to the biological degradation time scale and the equilibrium between different phases is always established. While this may be true in the laboratory soil experiments, the desorption from the old contaminated soil may be rate limiting. As will be argued later, these desorption rate-limited cases will actually benefit from the addition of surfactant by enhancing the desorption rate.

c) The presence of surfactant does not alter the physical activity of the biomass, i.e. the kinetic parameter of the degradation of aqueous-phase phenanthrene remains the same.

Assumption (a) was ensured by applying continuous mixing with a magnetic stirrer. Assumption (b) has been verified by Park and Jaffé (1993, 1994) for similar systems. In our case, sorption equilibrium was reached in less than one hour in all experiments. This can be considered instantaneous compared to the time required for the biodegradation. To verify the third assumption (c), an experiment was conducted with the same bacterial culture used for the biodegradation of phenanthrene, but degrading as carbon source a mixture of glucose and glutamic acid in the presence of different surfactant doses. These compounds are hydrophilic and will not partition into the micelles. Since surfactants will not affect their concentration in the aqueous phase, comparing their biodegradation in the presence and absence of a micellar surfactant phase is therefore a reasonable test to determine if the surfactant solution affects the bacterial population in a negative manner. As shown by the results in figure 13, the solutions containing the surfactant (at several doses above CMC), carbon source, and seed exerted the same amount of biochemical oxygen demand (BOD) as those without surfactant. This demonstrates that the presence of surfactant does not affect the biochemical activities of the biomass.

To simulate the biodegradation of phenanthrene in the presence of a surfactant solution, we substitute the substrate concentration in the Monod equation by an effective concentration. This effective concentration is defined as the sum of the concentration dissolved in water (C), the concentration bound to the monomer phase of the surfactant (C_{mon}), and the bioavailable concentration that has partitioned into the micellar phase (f x C_{mic}). Where f is the bioavailable fraction of the micelle bound contaminant (C_{mic}). The overall mass balance equations for the biodegradation of a hydrocarbon in the presence of a surfactant solution can then be written as follows:

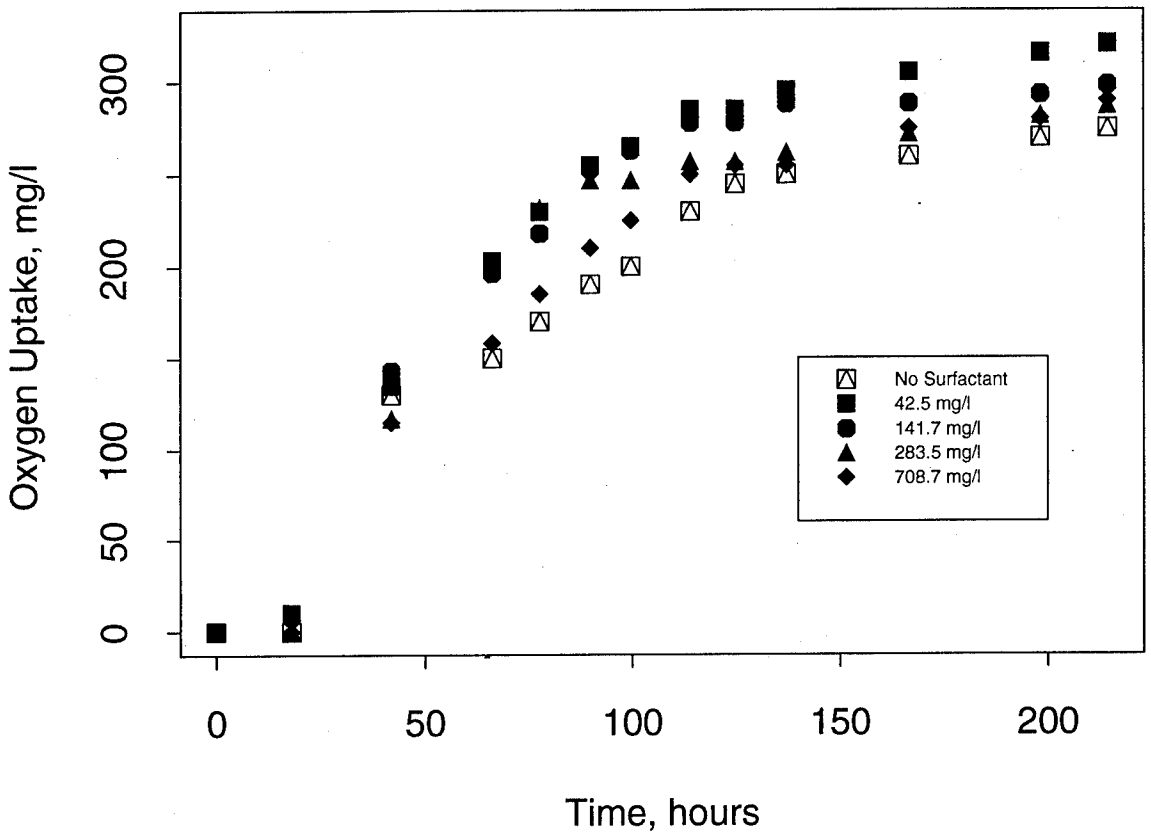
Contaminant (C) Balance:

$$R\frac{dC}{dt} = -\frac{m_m}{Y}\frac{\left(C + C_{mon} + f\,C_{mic}\right)}{K_S + \left(C + C_{mon} + f\,C_{mic}\right)}S$$

where,

$$R = 1 + K_{dg} + X_{mc}K_{mc} + X_{mn}K_{mn} + k_{oc}S$$

Figure 13: Biodegradation of BOD Standard in Presence of Triton N101



Biomass (S) Balance:

$$\frac{dS}{dt} = \mu_m \frac{(C + C_{mon} + fC_{mic})}{K_S + (C + C_{mon} + fC_{mic})} S - bS$$

Carbon Dioxide Production:

$$\frac{d[CO_2]}{dt} = \mu_m \left(\frac{1-Y}{Y}\right) \frac{(C + C_{mon} + fC_{mic})}{K_S + (C + C_{mon} + fC_{mic})} S + \text{Contribution from Endogenous Respiration}$$

With the exception of f, all of the parameters in the above equations have been determined previously from independent experiments. We can therefore simulate the biodegradation of phenanthrene in the presence of different surfactant concentrations, and by comparing these simulations against experimental data, we can determine to what degree the phenanthrene that has partitioned into the micellar phase is biodegradable. We will examine the following hypotheses:

a) The micellar phase contaminant is not directly bioavailable, and the degradation occurs from the water phase only. Mathematically this is expressed as f = 0.

b) The micellar phase contaminant is fully bioavailable, in addition to water phase contaminant. This is expressed as f = 1.

c) The micellar phase contaminant is only partially bioavailable. This means f has a value between 0 and 1.

The measured and modeled phenanthrene biodegradation is shown in figures 14a to 19b. The case of 'No Surfactant' illustrates the reliability of the parameter values by simulating the experimental results accurately (figures 14a and b). Second set with a surfactant concentration near the CMC imparted too much noise to the data (figures 15a and b) to draw any conclusion. As we increase the concentration of surfactant to about 4 times CMC, we start to observe the effect of the surfactant (figures 16a and b). There are four simulation lines representing, (a) degradation in water phase only (f = 0 and no C_{mon}), (b) degradation in water and monomer phase contaminant (f = 0 but C_{mon} added), (c) degradation of all three phases (f = 1 and C_{mon} added) and (d) degradation of water and micelle phase (f = 1 and no C_{mon}). Since K_{mn} is about an order of magnitude smaller than K_{mc} and X_{mc} is about 3 times X_{mn} (CMC), C_{mon} is a very small quantity. It gets even smaller as we increase the surfactant dose, because the extra surfactant increases X_{mc} whereas X_{mn} stays nearly constant. For this reason, lines (a) and (b) are close to each other, and both under predict the observed degradation rate. Lines (c) and (d) on the other hand, over predict the degradation rate. This indicates that along with water phase contaminant, only a fraction of the micellar phase contaminant is degraded. This effect is more pronounced as we increased the dose of surfactant to ~8 times CMC (figures 17a and b), ~15 times CMC (figures 18a and b) and ~20 times CMC (figures 19a and b). This points to the hypothesis (c) above that f is greater than zero but less than one.

Results shown in figures 16a through 19b can be simulated with reasonable accuracy using different values of f (figures 20a through 23b). The factor f depends on the surfactant type, dose and biomass concentration. The later dependency is illustrated in figures 24a through 25b where, the experiment was performed with the a surfactant dose of 283.5 mg/l (figures 17a and 17b) but with two different biomass concentrations. The inverse dependency of f as a function of the surfactant dose is summarized by the results shown in figure 26.

Figure 14a: Simulation(line) of the Phenanthrene Concentration for No Surfactant

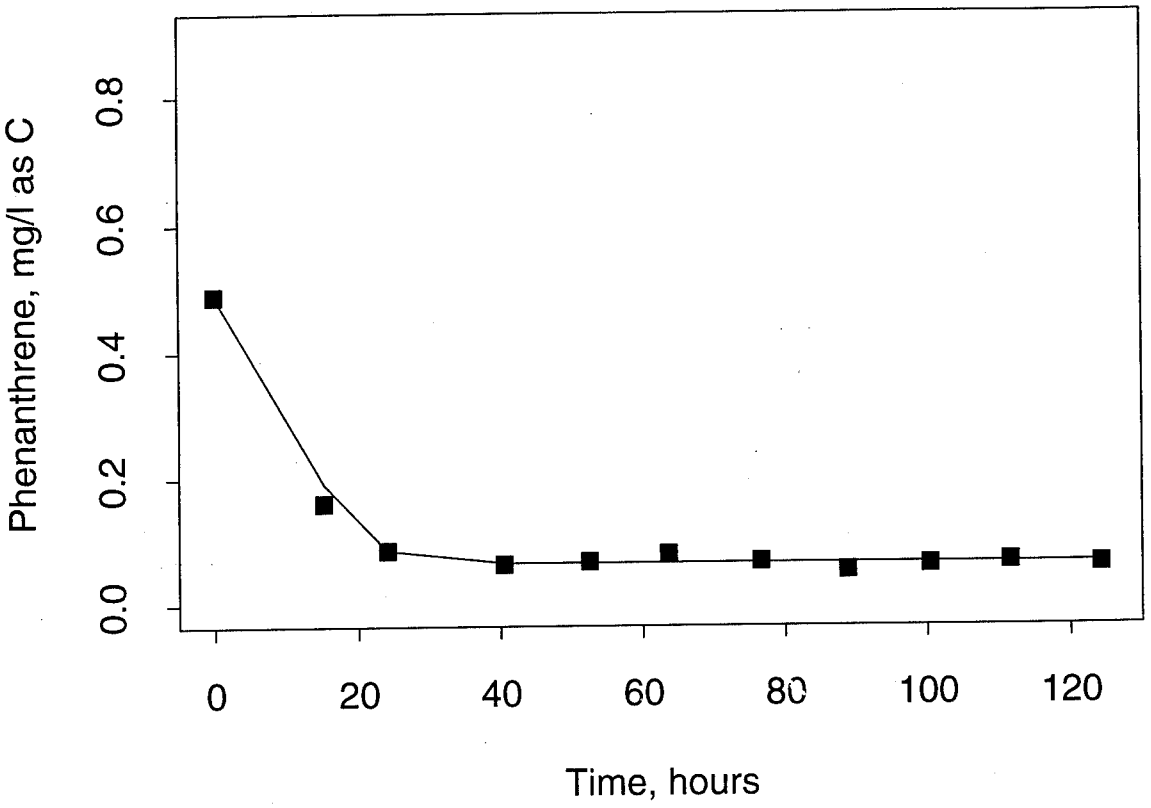


Figure 14b: Simulation(line) of the Carbon Dioxide Concentration for No Surfactant

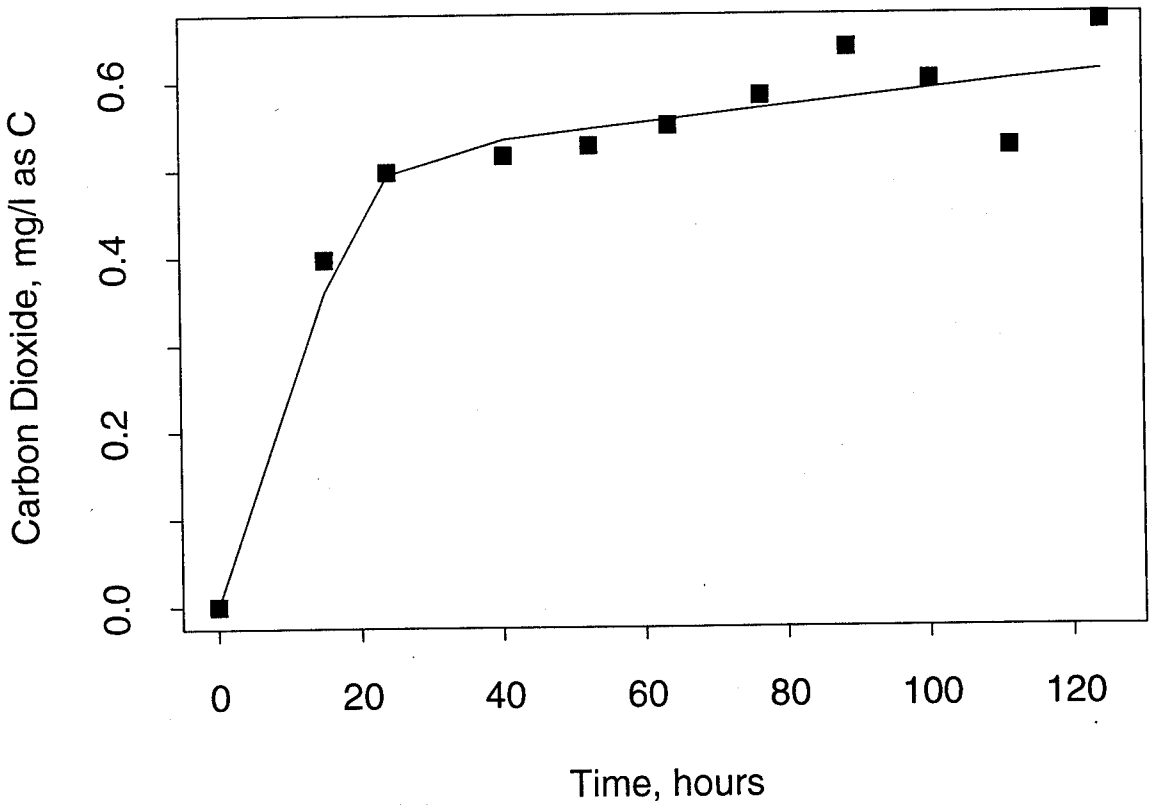
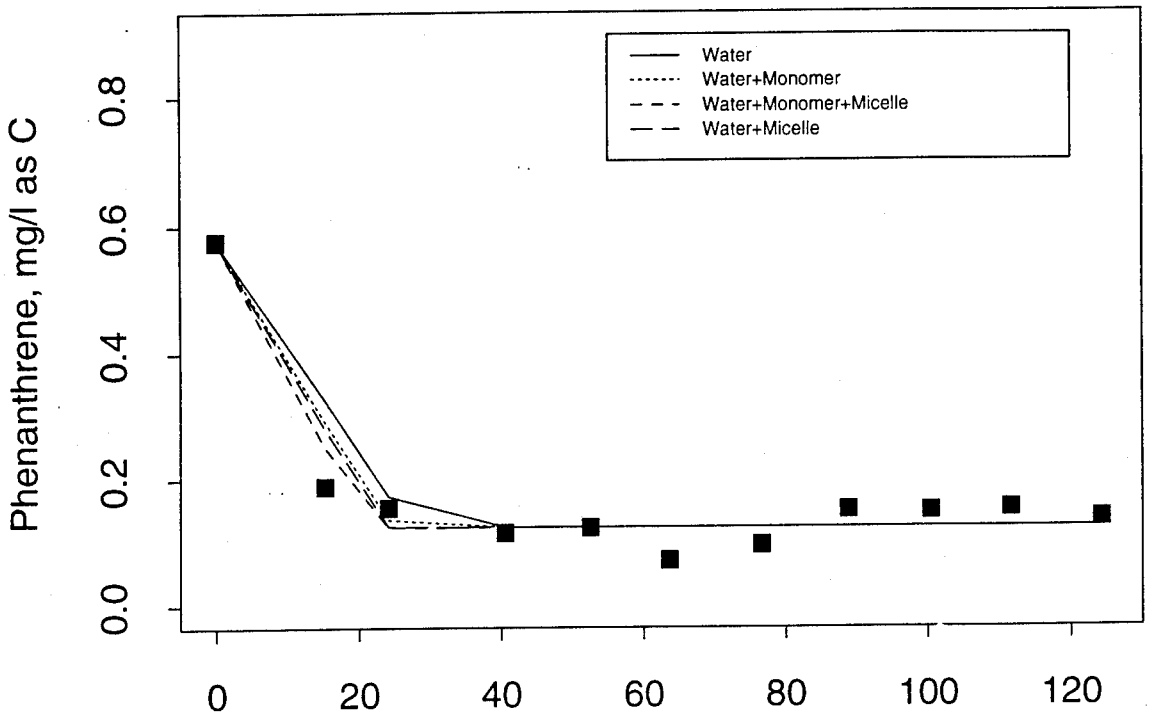
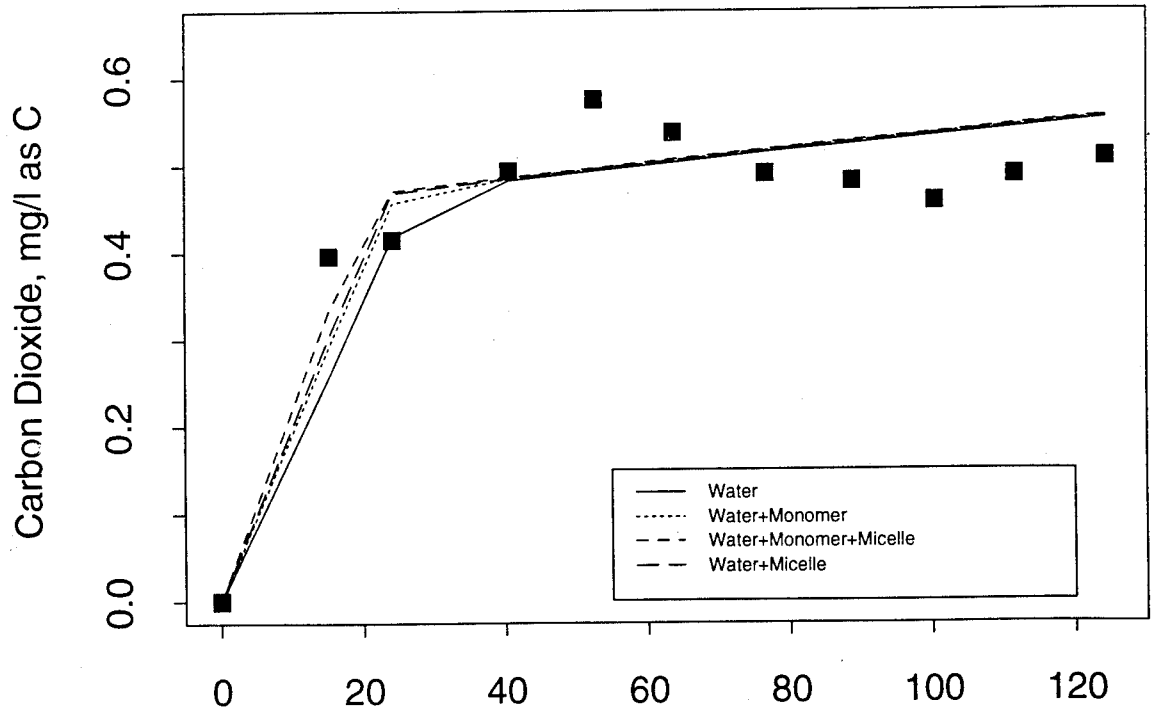


Figure 15a: Simulation(line) of the Phenanthrene Concentration for 42.5 mg/l of Triton N101



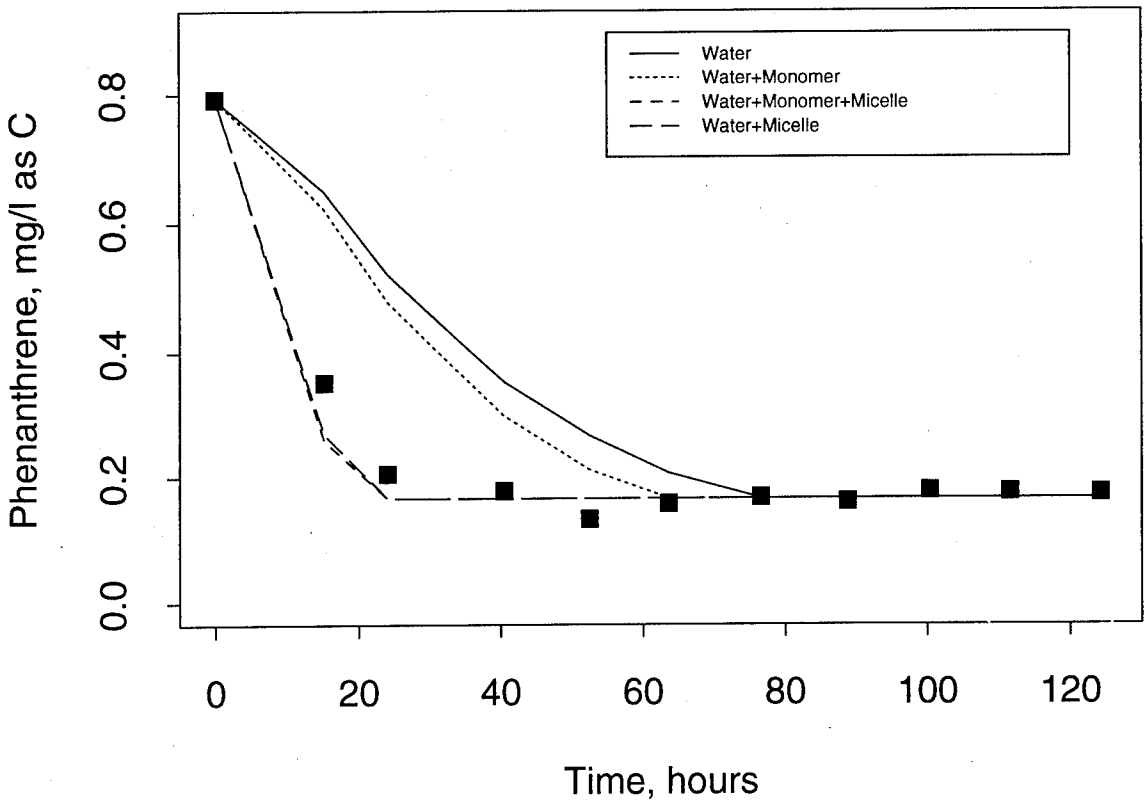
Legend shows the Degradation of the Contaminants in Different Phases

Figure 15b: Simulation(line) of the Carbon Dioxide Concentration for 42.5 mg/l of Triton N101



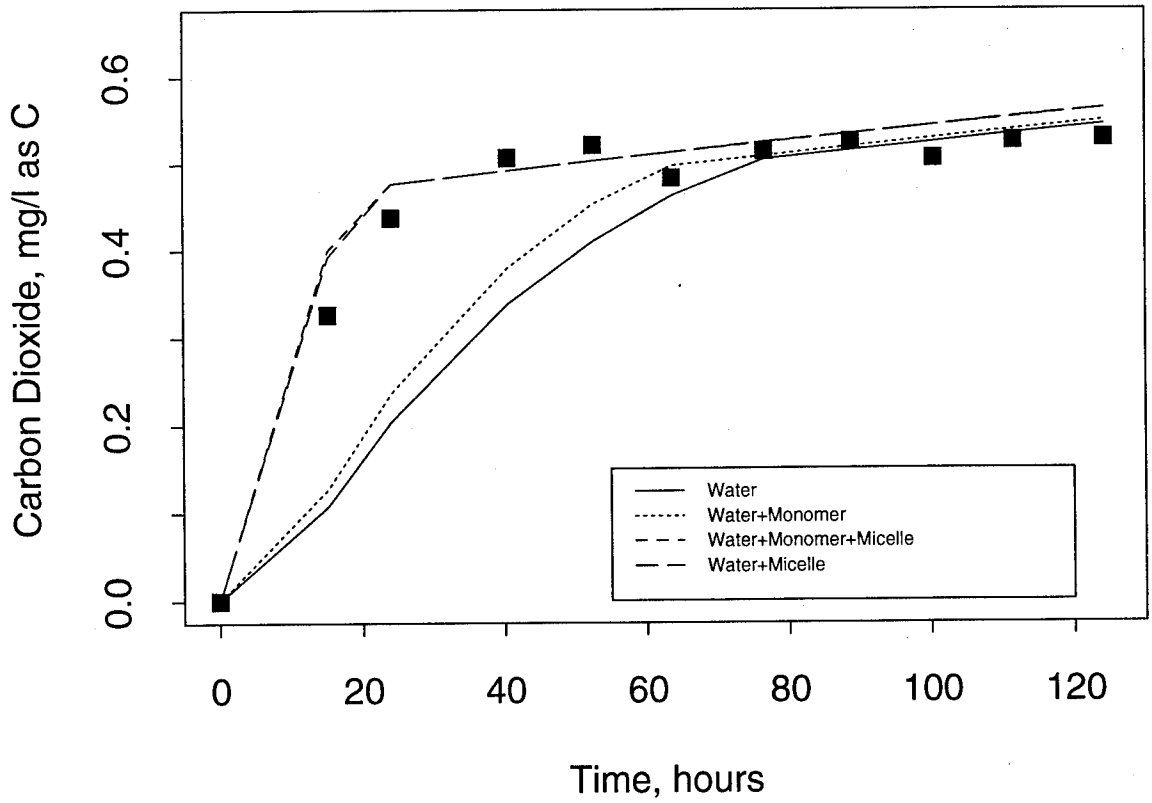
Legend shows the Degradation of the Contaminants in Different Phases

Figure 16a: Simulation(line) of the Phenanthrene Concentration for 141.7 mg/l of Triton N101



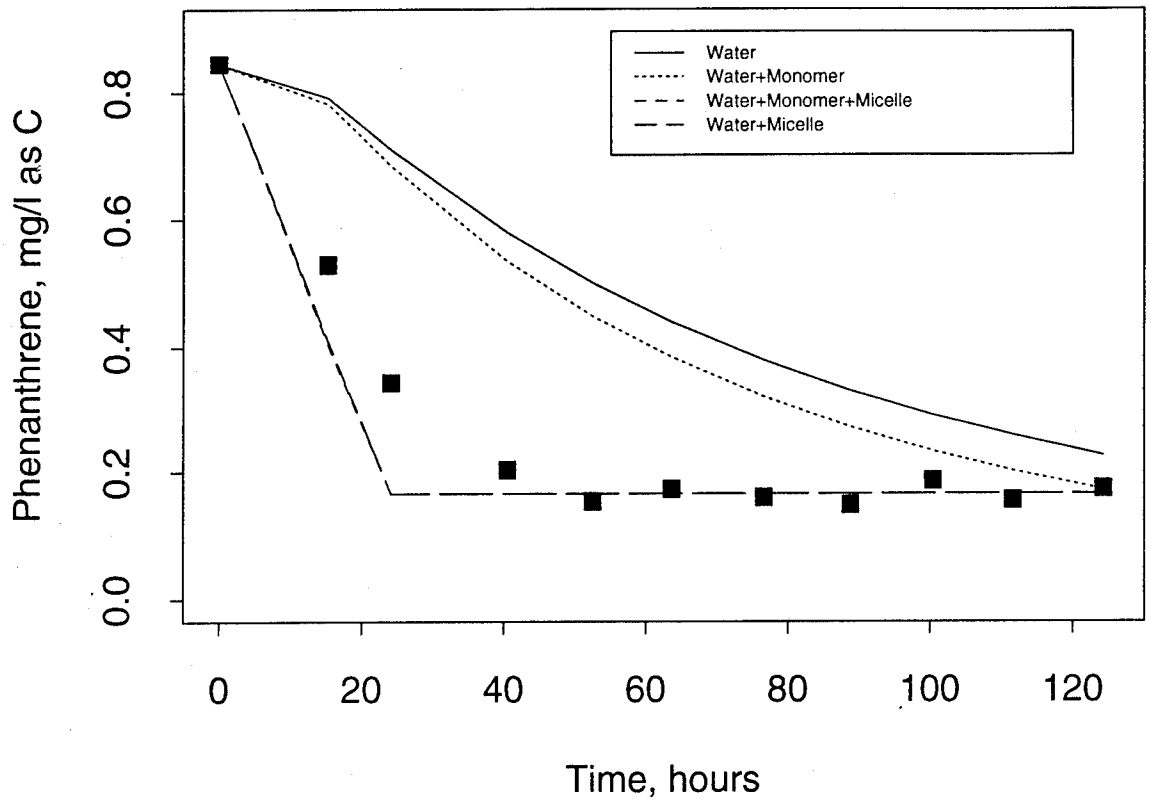
Legend shows the Degradation of the Contaminants in Different Phases

Figure 16b: Simulation(line) of the Carbon Dioxide Concentration for 141.7 mg/l of Triton N101



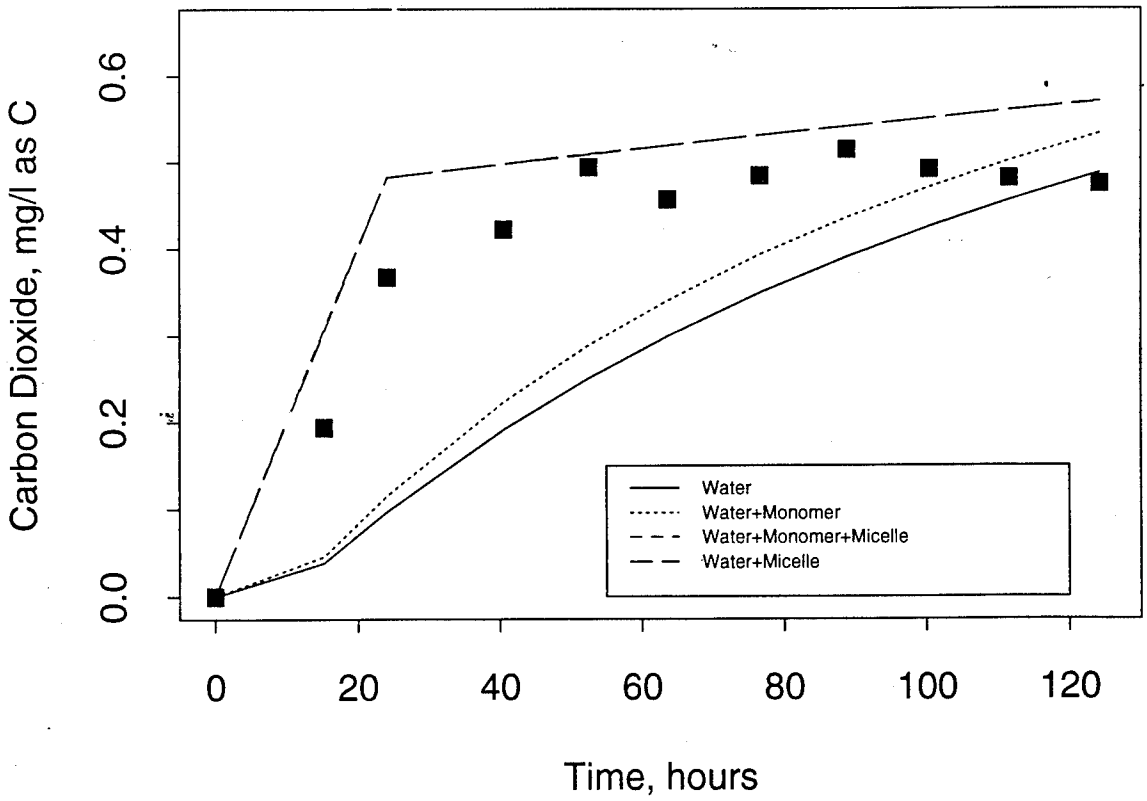
Legend shows the Degradation of the Contaminants in Different Phases

Figure 17a: Simulation(line) of the Phenanthrene Concentration for 283.5 mg/l of Triton N101



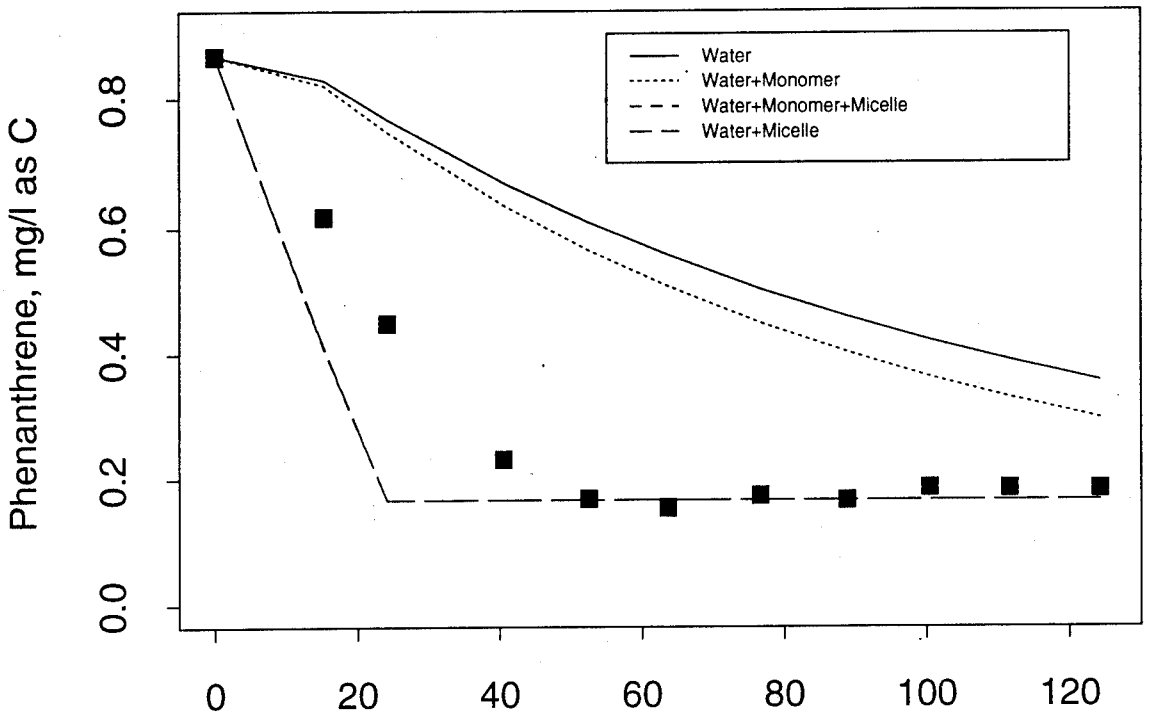
Legend shows the Degradation of the Contaminants in Different Phases

Figure 17b: Simulation(line) of the Carbon Dioxide Concentration for 283.5 mg/l of Triton N101



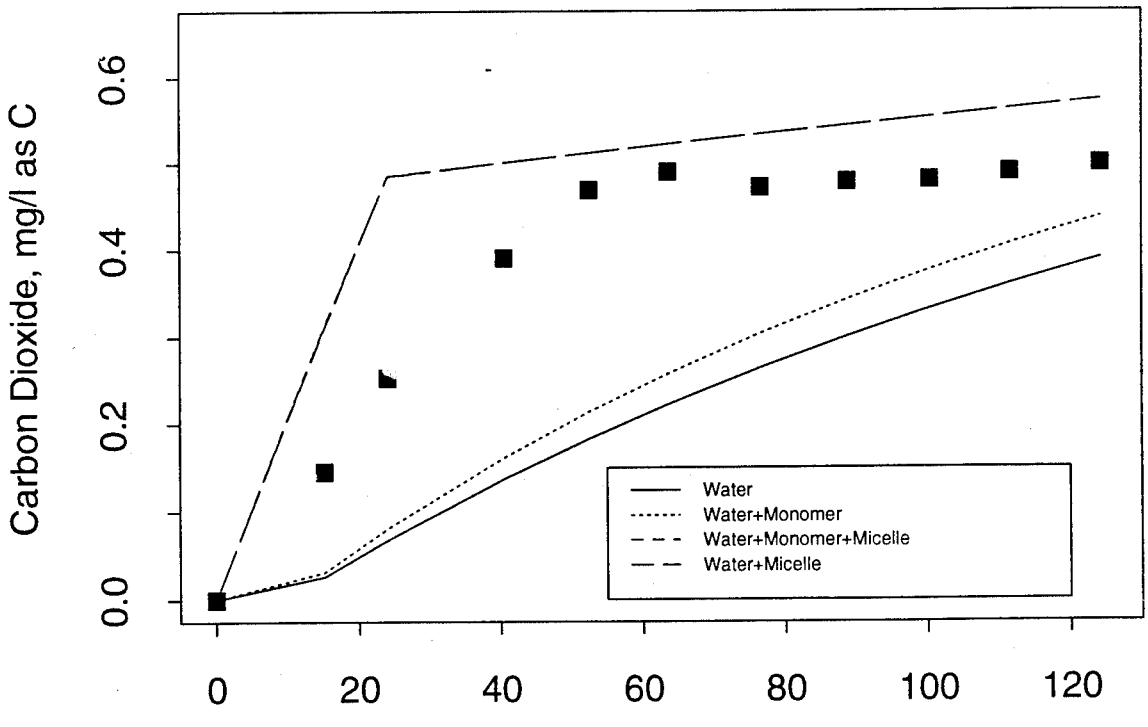
Legend shows the Degradation of the Contaminants in Different Phases

Figure 18a: Simulation(line) of the Phenanthrene Concentration for 425.2 mg/l of Triton N101



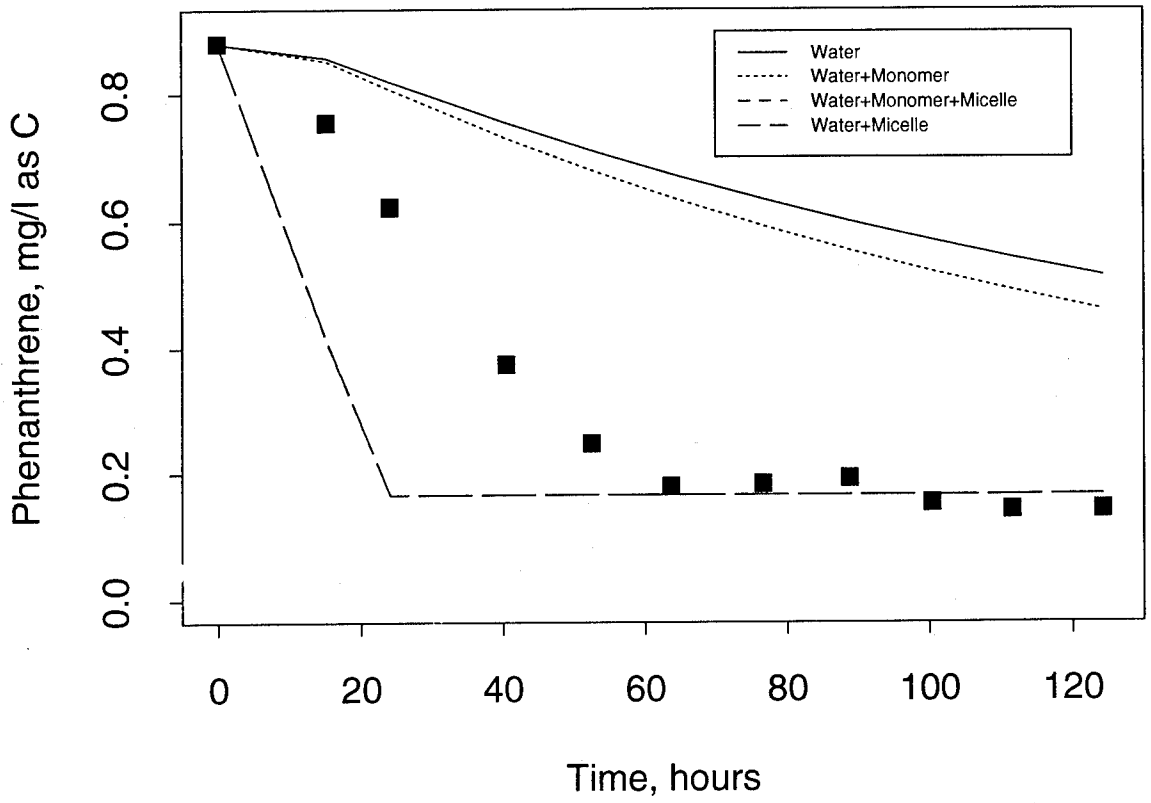
Legend shows the Degradation of the Contaminants in Different Phases

Figure 18b: Simulation(line) of the Carbon Dioxide Concentration for 425.2 mg/l of Triton N101



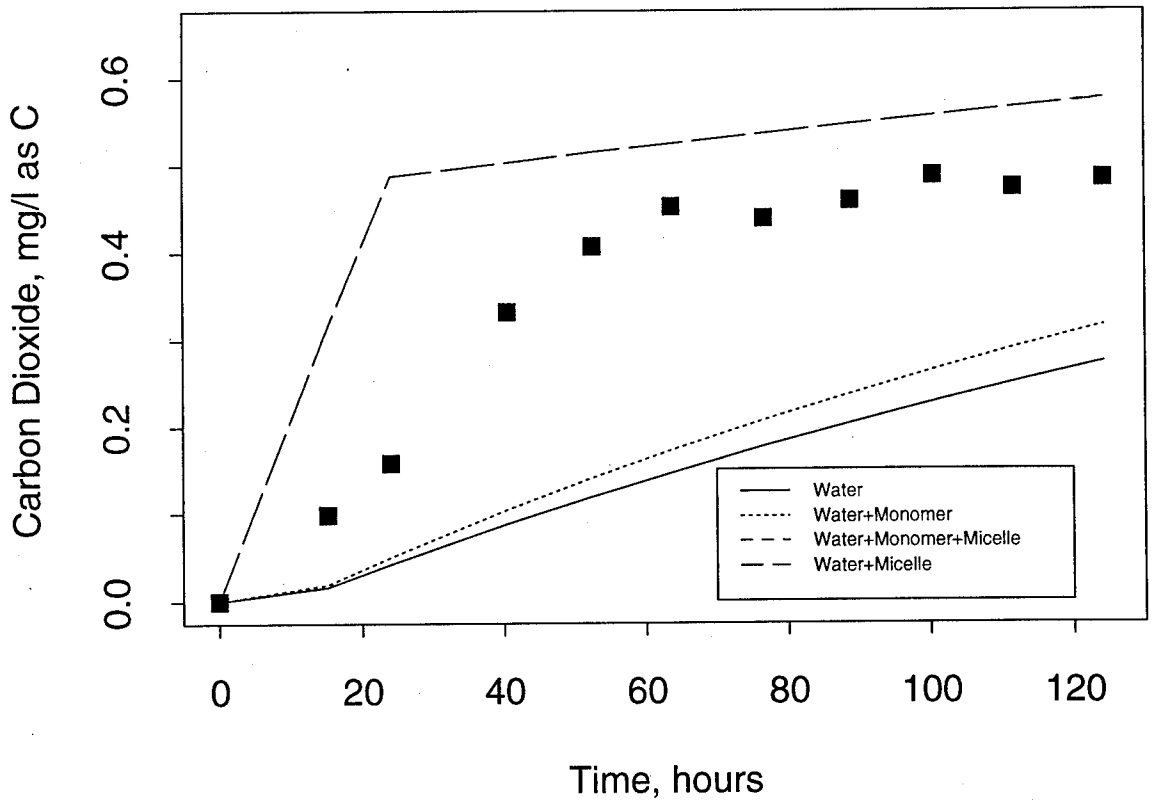
Legend shows the Degradation of the Contaminants in Different Phases

Figure 19a: Simulation(line) of the Phenanthrene Concentration for 708.7 mg/l of Triton N101



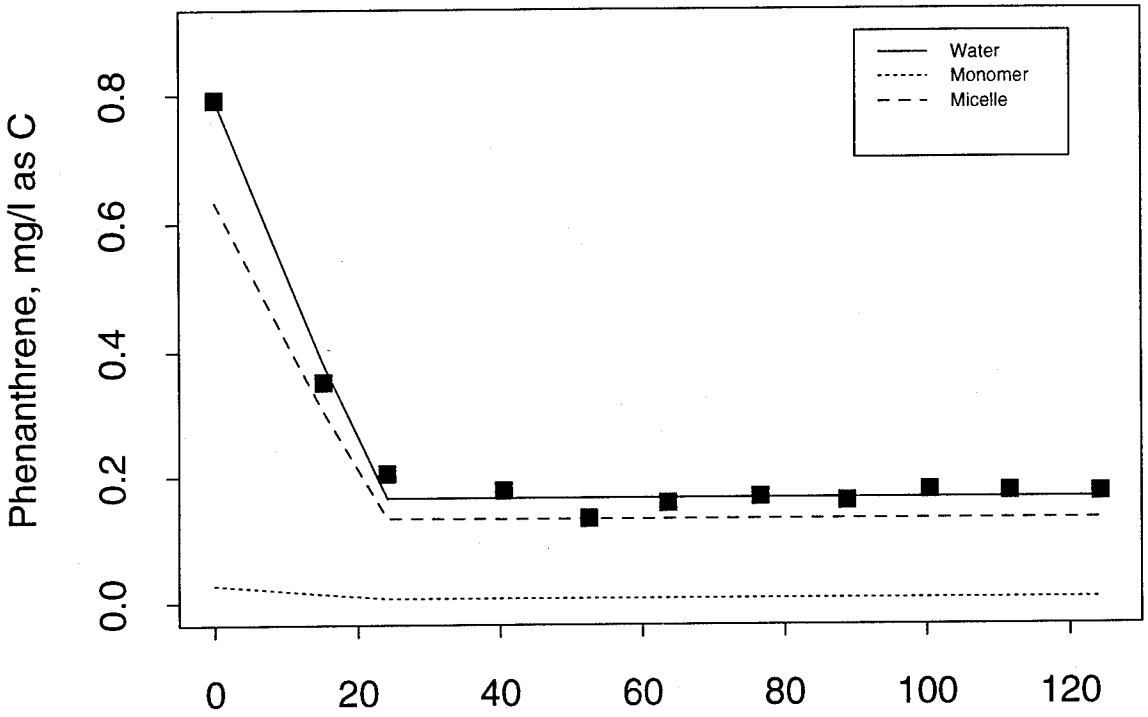
Legend shows the Degradation of the Contaminants in Different Phases

Figure 19b: Simulation(line) of the Carbon Dioxide Concentration for 708.7 mg/l of Triton N101



Legend shows the Degradation of the Contaminants in Different Phases

Figure 20a: Simulation(line) of the Phenanthrene Concentration for 141.7 mg/l of Triton N101 with f=0.55



Legend Shows Contaminant Concentration in Different Phases

Figure 20b: Simulation(line) of the Carbon Dioxide Concentration for 141.7 mg/l of Triton N101 with f=0.55

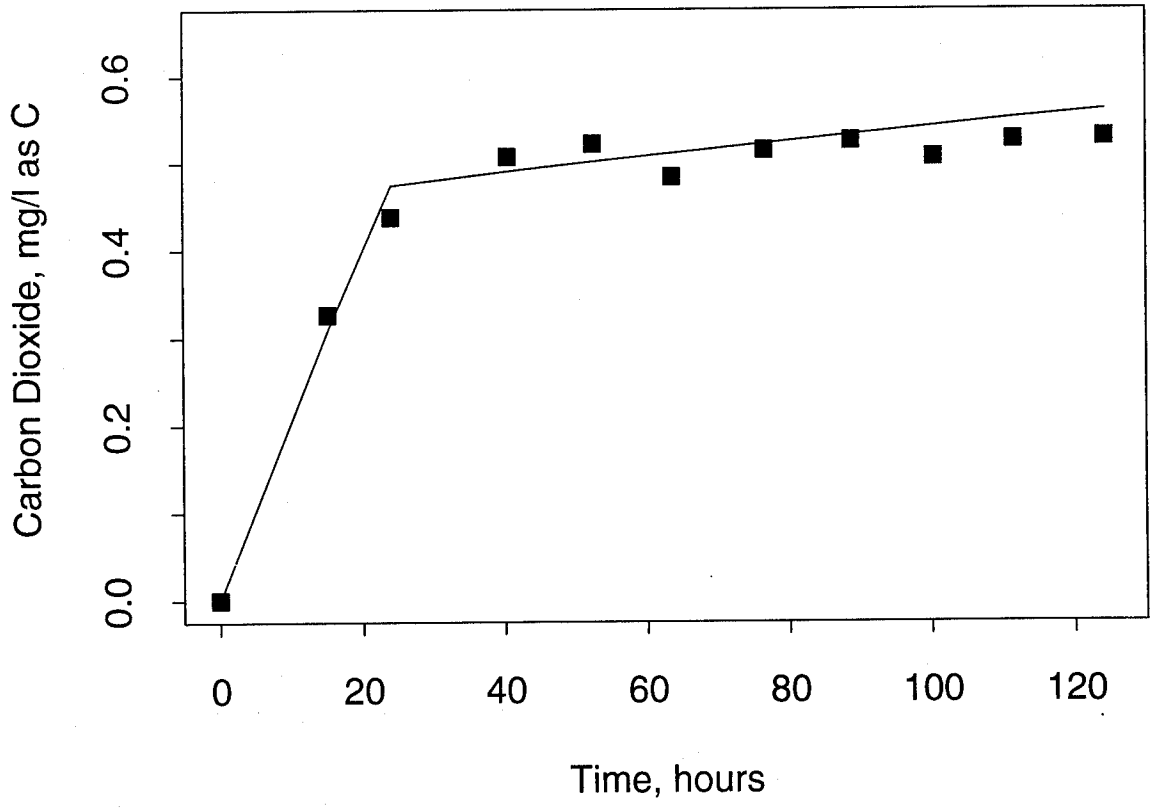
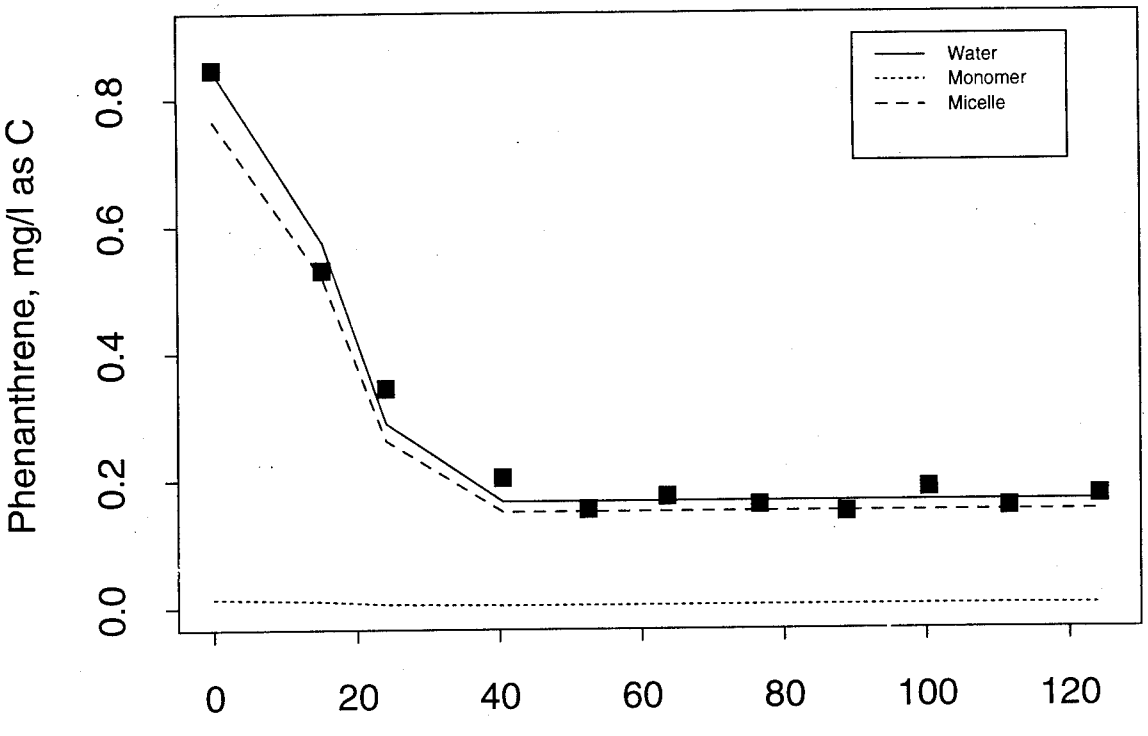


Figure 21a: Simulation(line) of the Phenanthrene Concentration for 283.5 mg/l of Triton N101 with f=0.45



Legend Shows Contaminant Concentration in Different Phases

Figure 21b: Simulation(line) of the Carbon Dioxide Concentration for 283.5 mg/l of Triton N101 with f=0.45

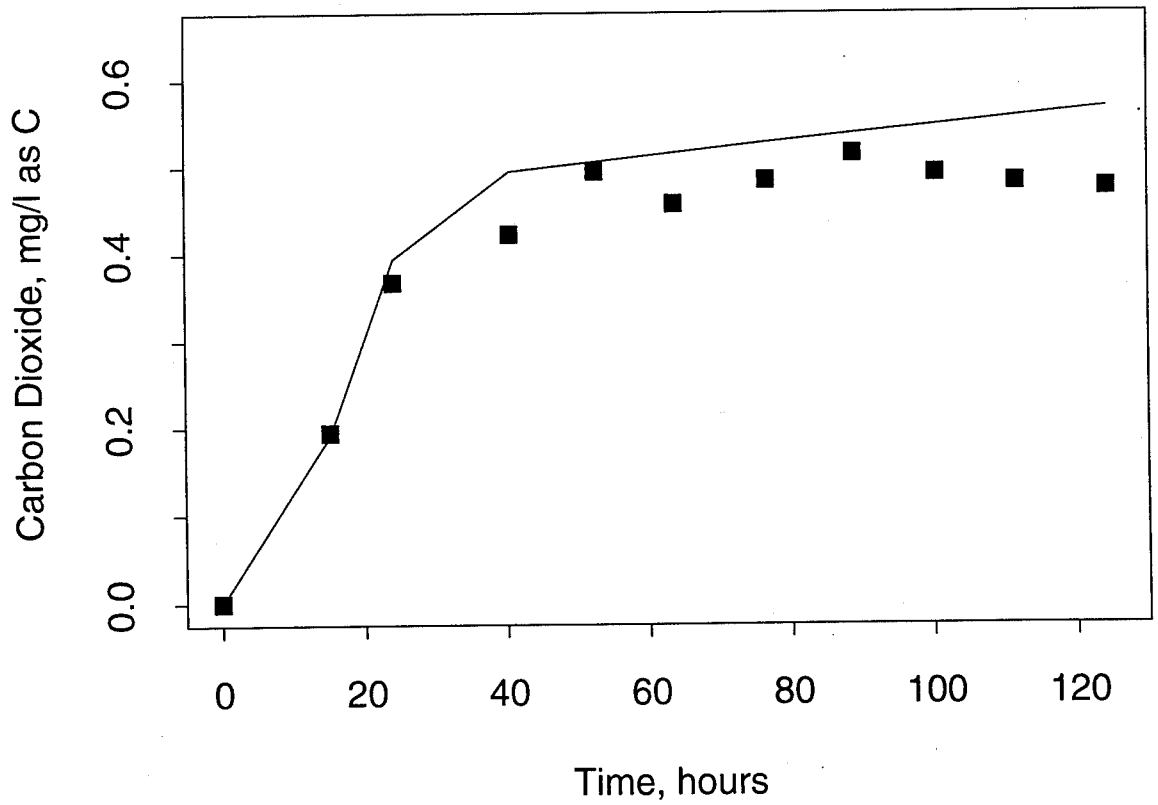
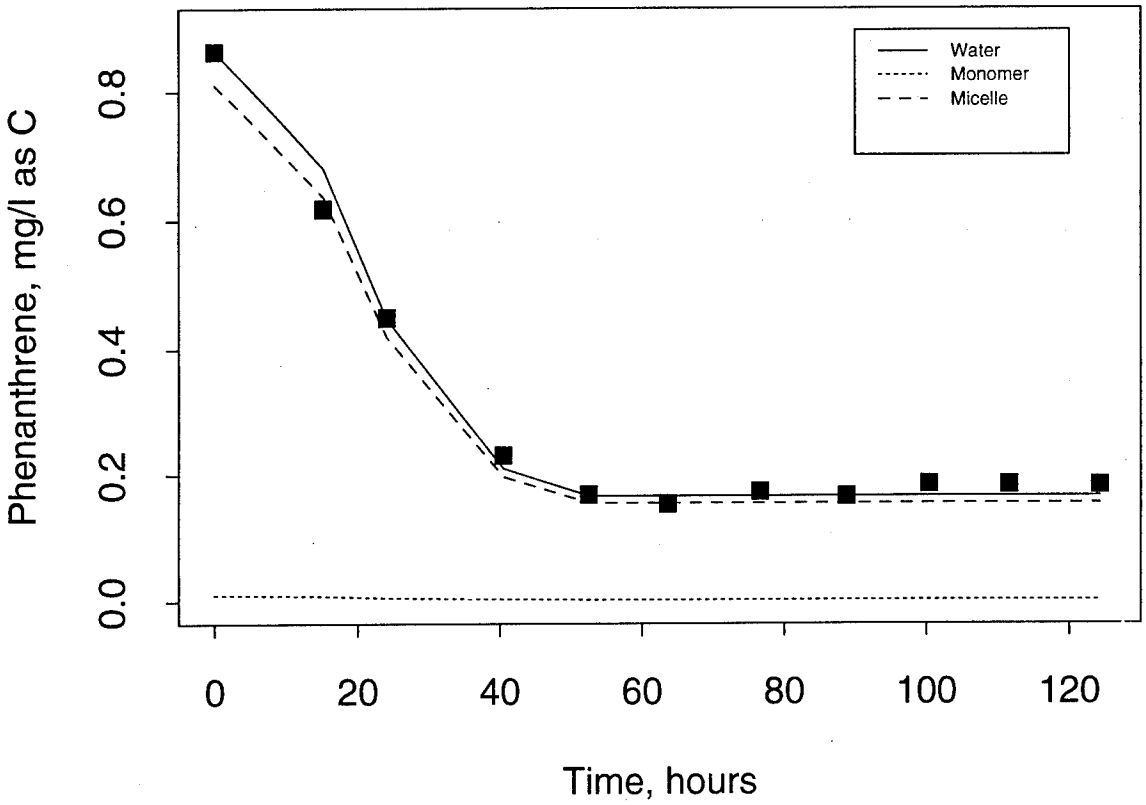


Figure 22a: Simulation(line) of the Phenanthrene Concentration for 425.2 mg/l of Triton N101 with f=0.25



Legend Shows Contaminant Concentration in Different Phases

Figure 22b: Simulation(line) of the Carbon Dioxide Concentration for 425.2 mg/l of Triton N101 with f=0.25

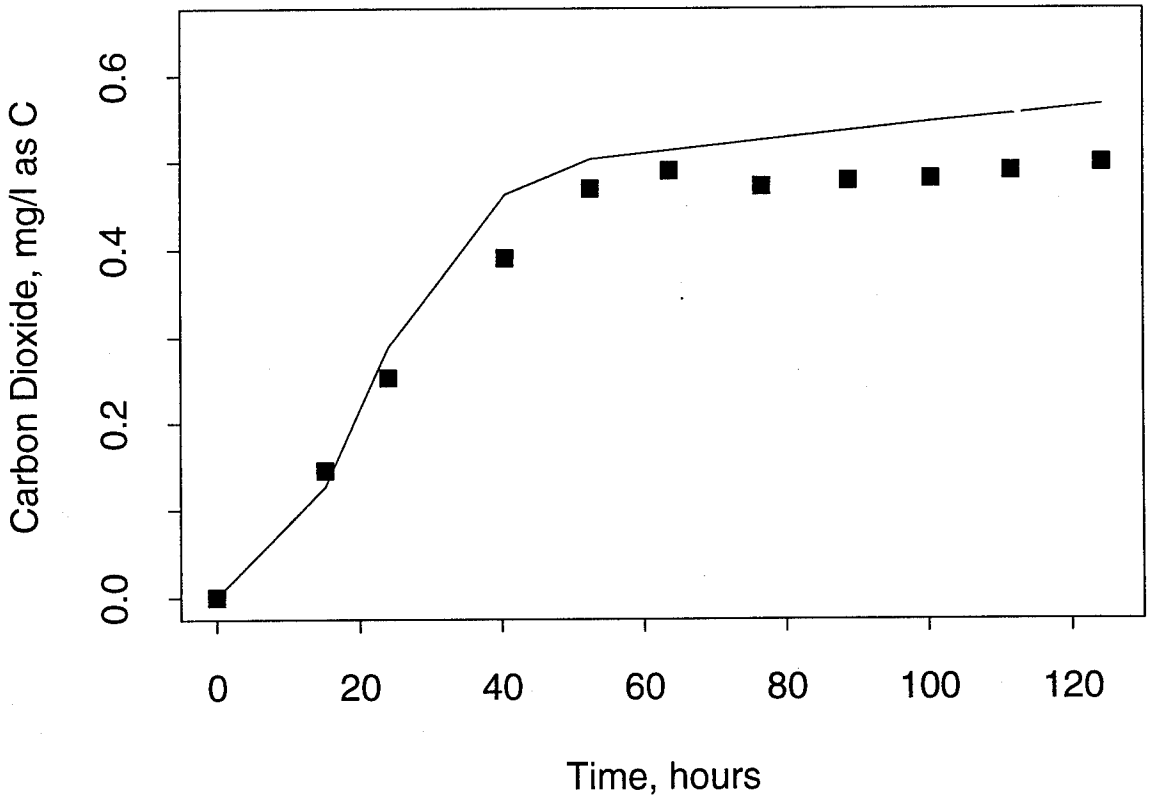
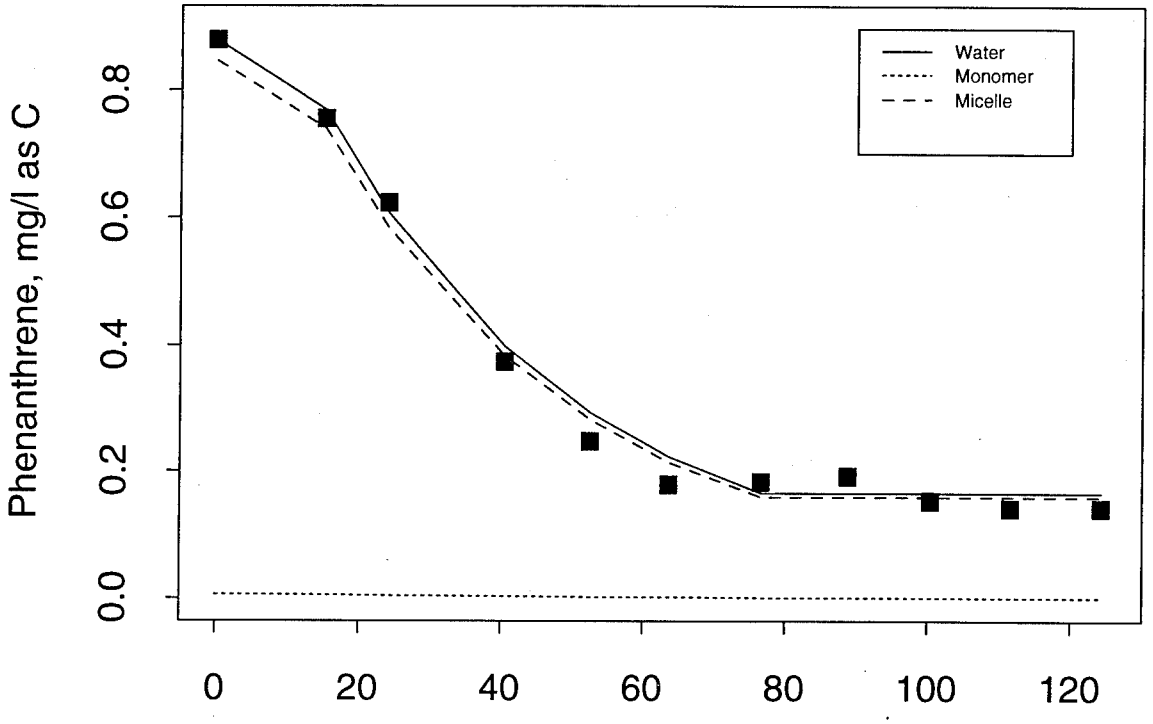


Figure 23a: Simulation(line) of the Phenanthrene Concentration for 708.7 mg/l of Triton N101 with f=0.13



Legend Shows Contaminant Concentration in Different Phases

Figure 23b: Simulation(line) of the Carbon Dioxide Concentration for 708.7 mg/l of Triton N101 with f=0.13

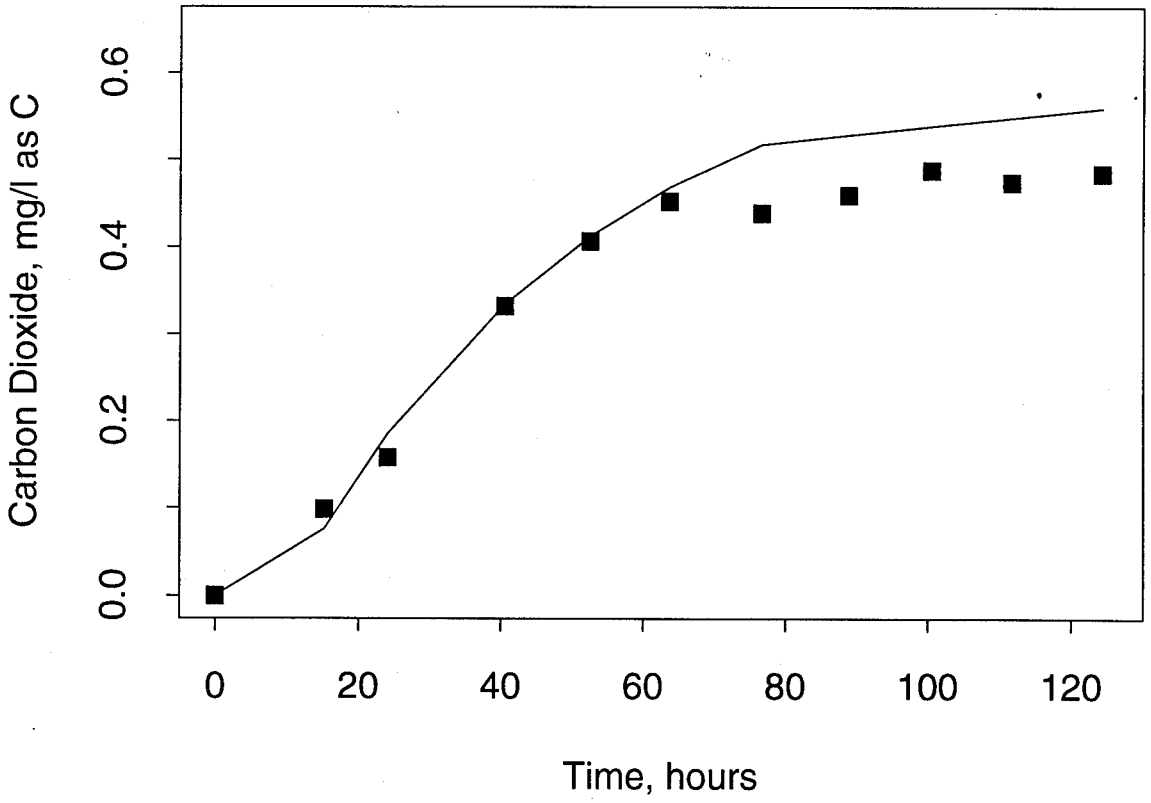
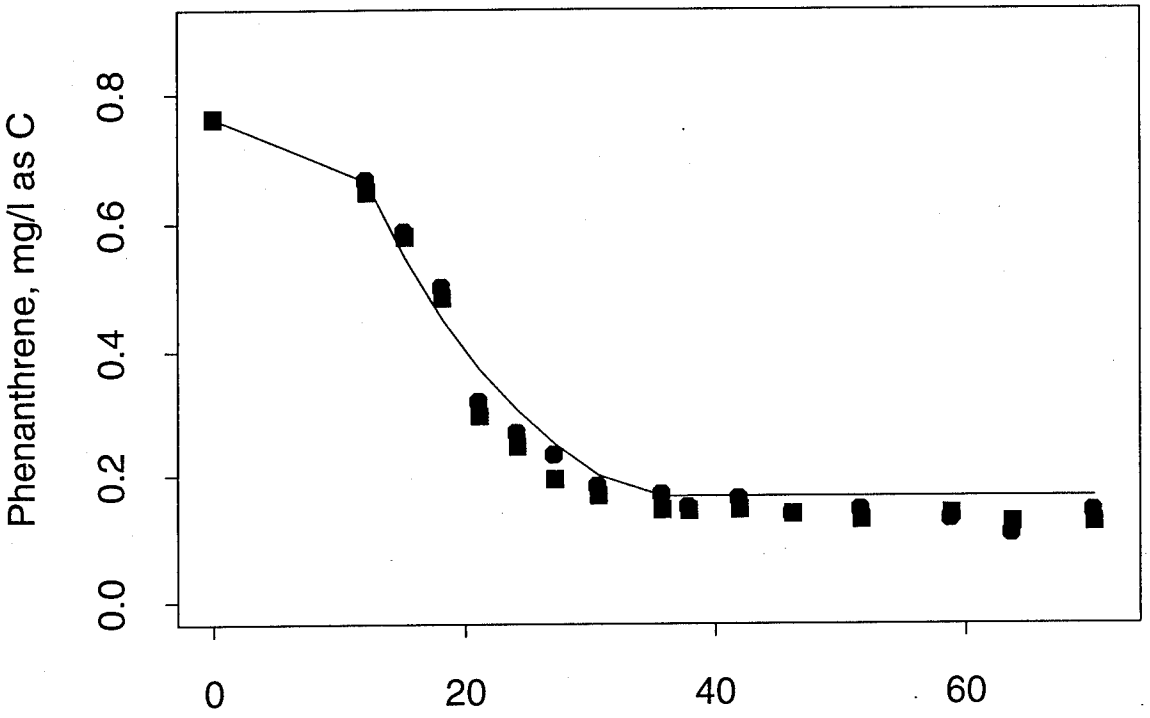
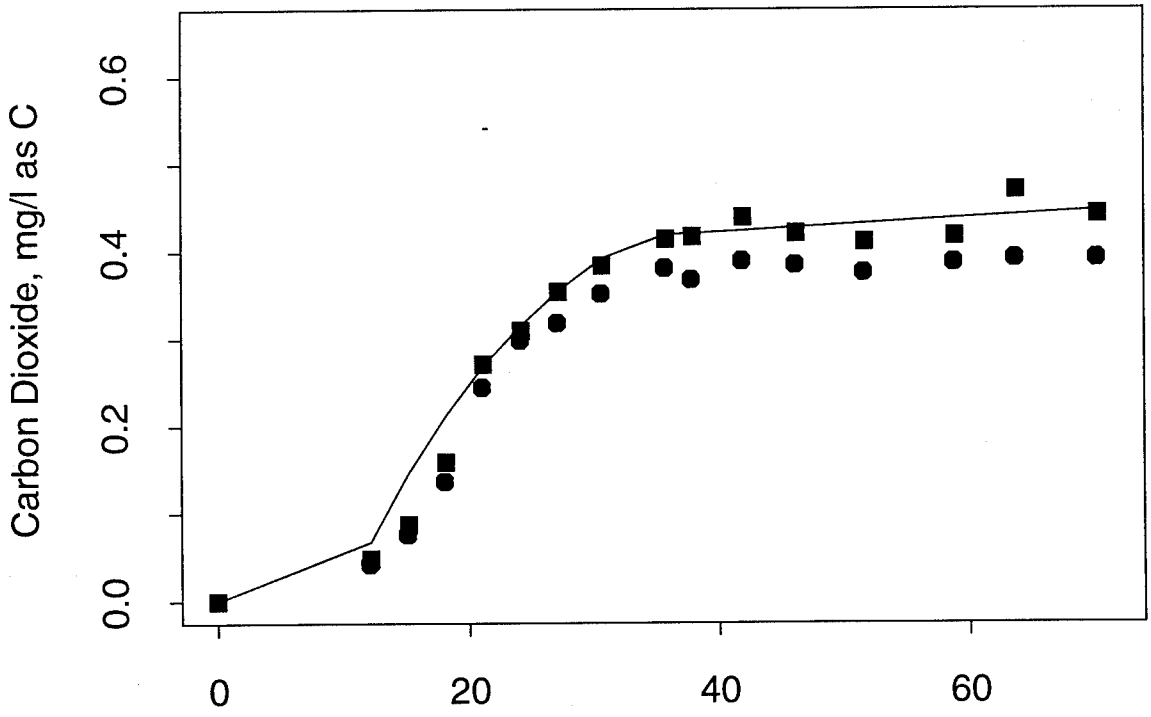


Figure 24a: Simulation(line) of the Phenanthrene Concentration for 283.5 mg/l of Triton N101 with f=0.7



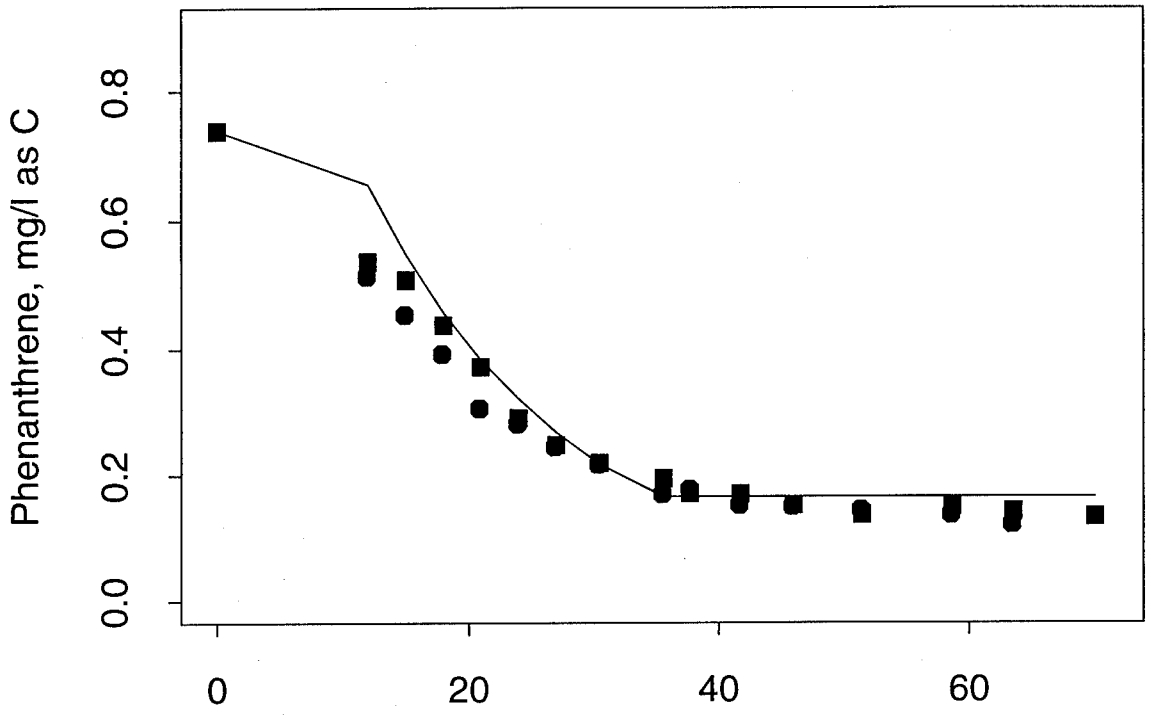
Initial Biomass Concentration is 28.96 mg/l as Carbon

Figure 24b: Simulation(line) of the Carbon Dioxide Concentration for 283.5 mg/l of Triton N101 with f=0.7



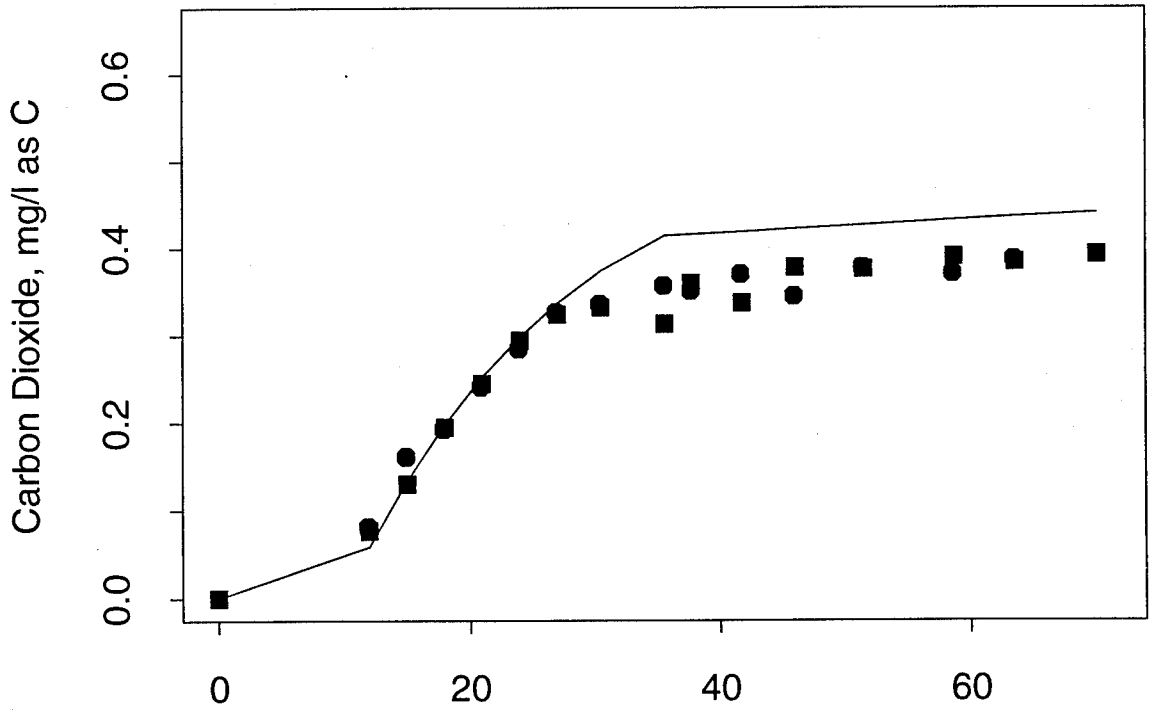
Initial Biomass Concentration is 28.96 mg/l as Carbon

Figure 25a: Simulation(line) of the Phenanthrene Concentration for 283.5 mg/l of Triton N101 with f=0.25



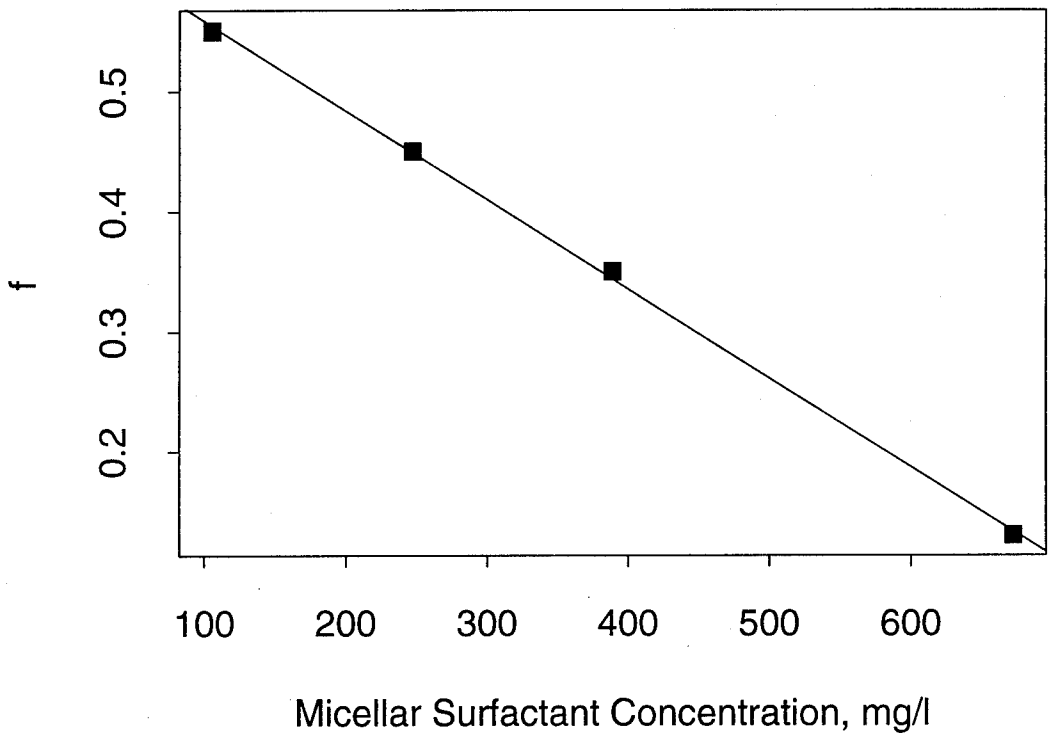
Initial Biomass Concentration is 60.0 mg/l as Carbon

Figure 25b: Simulation(line) of the Carbon Dioxide Concentration for 283.5 mg/l of Triton N101 with f=0.25



Initial Biomass Concentration is 60.0 mg/l as Carbon

Figure 26: Variation of the factor(f) with Micellar Surfactant concentration



Biodegradation in Presence of Soil and Surfactant

^{14}C-phenanthrene was allowed to sorb onto soil for 12 hours before adding the surfactant and seed. Results of the biodegradation of the phenanthrene in the presence of soil are shown in figure 27a. In this case, the addition of the surfactant does not show a significant effect on the degradation rate of phenanthrene when compared to that without surfactant addition. The soil has a high organic carbon content (Table 2) and is capable of sorbing a significant amount of surfactant as shown by the sorption equilibrium isotherm (figure 28). This essentially increases the carbon content of the soil and thus, the sorption capacity of phenanthrene by the surfactant-coated soil, which leads to a net decrease of the aqueous and micellar-phase phenanthrene (figure 27b).

When the desorption rate of a hydrocarbon from soil is the limiting factor in its biodegradation, the addition of surfactants can increase the desorption rate significantly. In this case the degradability of the micelle phase contaminant may have a larger impact.

These results have shown the importance in selecting a surfactant that does not sorb strongly onto soils in order to use surfactants to enhance the bioremediation of contaminated soils. It should be noted that a lower soil-carbon content will yield less surfactant sorption onto the soil.

Figure 27a: Carbon Dioxide Production in Presence of 50 g/l Soil

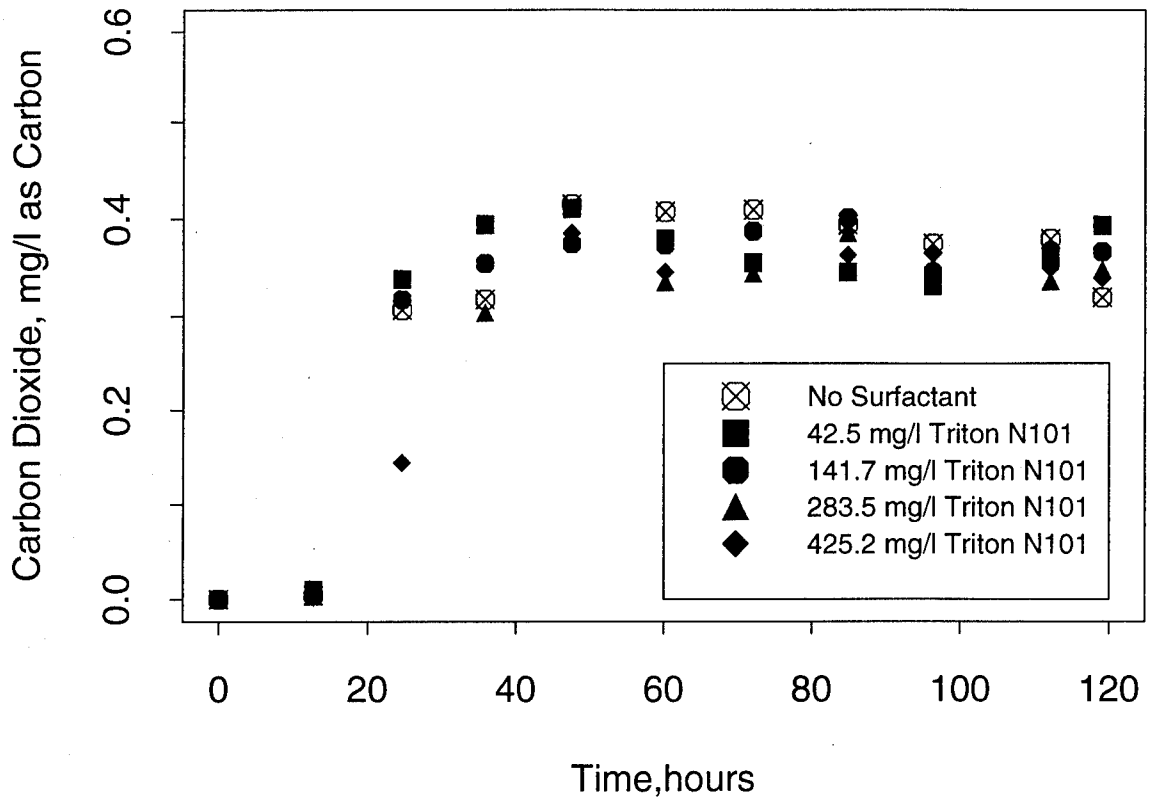


Figure 27b: Phenanthrene in Solution in Presence of 50 g/l Soil

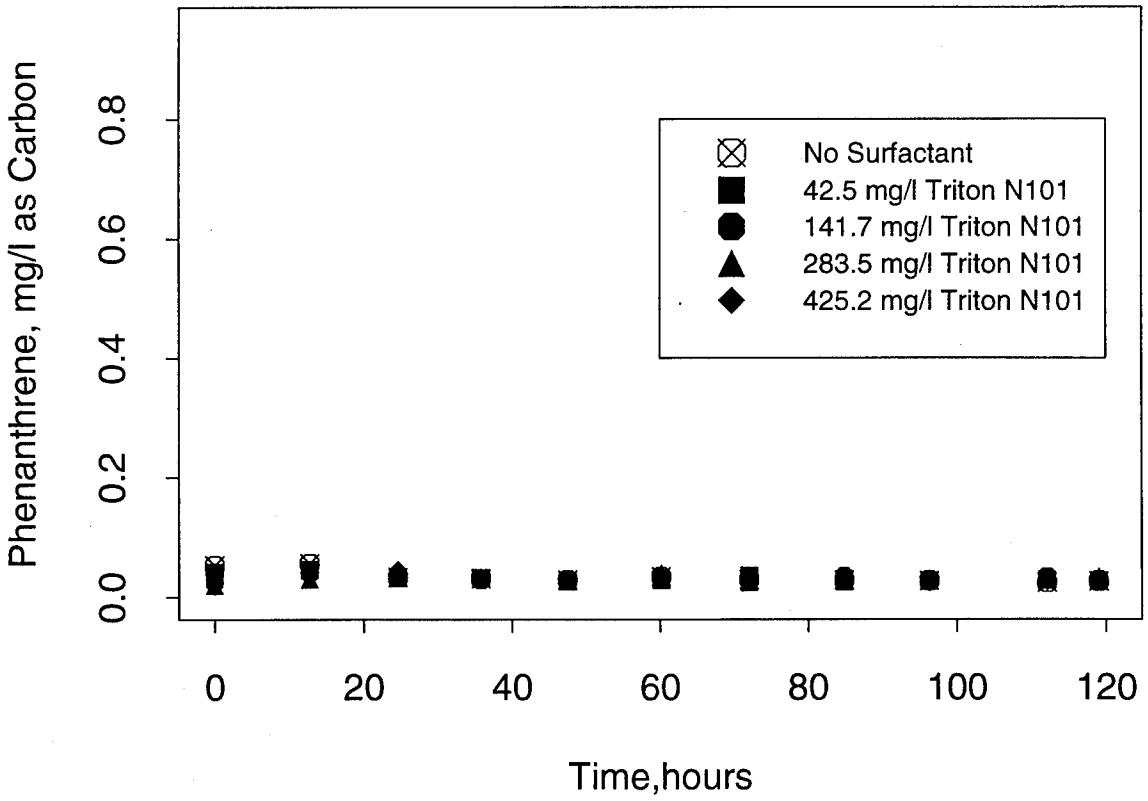
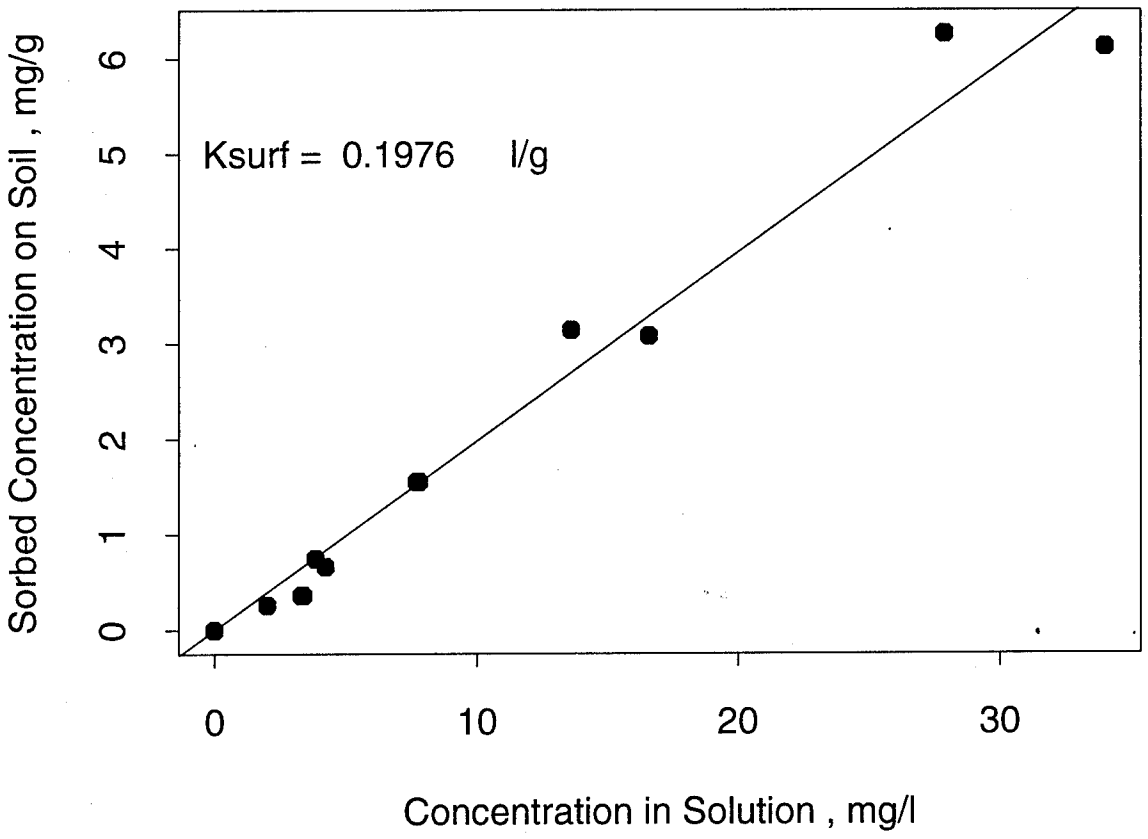


Figure 28: Sorption Equilibrium of Triton N101 on Soil



Conclusions

The following general conclusions can be drawn from the results shown and discussed above:

1) Phenanthrene solubilized in the core of surfactant micelles can be biodegraded by mixed bacterial cultures.

2) The degree to which the micellar phase phenanthrene is degradable, is a function of the surfactant type, the surfactant dose, and the biomass concentration.

3) Inhibition of the biodegradation of phenanthrene in the presence of surfactants above their CMC is dependent on the type of surfactant.

4) The use of surfactants to enhance the biodegradation of low-solubility hydrocarbons in soil-slurry reactors may not be practical for soils with a high organic carbon content, and sorbing non-ionic surfactants.

5) A significant portion of phenanthrene can be solubilized by the biomass itself.

6) The addition of surfactants increased the non-degradable residual in the batch reactors.

Applications and Future Research Directions

1) Surfactants solutions above their critical micelle concentration can increase significantly the efficiency of soil-washing schemes. This is true for soils contaminated with a non-aqueous-phase liquid (NAPL) and/or residual contamination. Surfactant aided soil washing is not economically feasible unless the surfactant can be recycled. In this research, it was shown that the contaminant can be degraded without readily degrading the surfactant. This demonstrates the feasibility of an above ground bioreactor/regeneration facility for the spent wash-solution.

2) A major barrier for the efficient operation of surfactant-enhanced soil-slurry bioreactor is the sorption of the surfactant onto the soil, as demonstrated above. For this purpose, future research should be directed to identify surfactants which sorb less onto soils yet allow for the biodegradation of the micellar-phase hydrocarbon.

3) The factor f, described in the formulation of the degradation kinetics for the micellar phase hydrocarbon has to be generalized for different surfactant types and doses. To allow for a proper design and operation of surfactant-enhanced bioremediation schemes, future research should focus on determining theoretical or semi-empirical formulations that can describe f in terms of surfactant and contaminant variables.

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Section B

Solubilization and Biodegradation of Excess Octadecane in the Presence of Nonionic Surfactants

Abstract

Solubilization and biodegradation of octadecane was studied in the presence of four nonionic surfactants: Brij 35, Corexit 0600, Triton X-114, and Tween 40. Octadecane (solubility = 0.007 mg/L in water at 25°C) is a constituent in the paraffin fraction of petroleum and is often found in contaminated soils. Brij 35, Corexit 0600, Triton X-114, and Tween 40 are four commercial surfactants which represent some of the major classes of nonionic surfactants.

Batch studies were carried out in shake flasks to measure total and molecular solubility enhancement of octadecane in solutions of surfactants below and above their critical micelle concentration (CMC). Total solubilities were measured after centrifugation to remove excess solid phase octadecane. In addition, solute size distributions were determined from concentration measurements after sequential filtration through a 0.2 μm filter and molecular filters.

Results indicate that surfactants induce enhancement of octadecane solubility, even though the increase in solubility is primarily the result of octadecane-surfactant aggregates rather than molecular dispersions of octadecane. Corexit 0600 and Triton X-114 were most efficient in enhancing octadecane solubility, while Tween 40 was least efficient.

Biodegradation experiments were carried out in batch tests with oxygen utilization and protein production measured as indication of growth. Experiments were carried out in the presence and absence of Jordan sand, a low organic content silica mineral, at two surfactant concentrations, one below and one above each surfactant's CMC. Octadecane was added in the particulate form in the tests without soil, and as a coated film on the sand in the tests with soil.

Addition of surfactant slightly enhanced octadecane biodegradation rates in the case of two surfactants, namely, Corexit 0600 and Tween 40. However, for all surfactants tested, increasing the surfactant concentration from below to above the CMC did not further significantly enhance octadecane uptake rates.

The introduction of octadecane as a coating on Jordan sand appeared to enhance octadecane utilization rate more than the addition of surfactant. Evidently the coated octadecane increased substrate bioavailability by creating a larger surface area exposure than was achieved by surfactant wetting of the particulate octadecane.

Corexit 0600 and Tween 40 were taken up readily by the octadecane-acclimated culture. However, Brij 35 and Triton X-114 were not utilized during the course of the experiments. None of the tested surfactants appeared to inhibit octadecane utilization except for Triton at the concentration above its CMC.

Adsorption of the four surfactants on Jordan sand and octadecane-coated Jordan sand was investigated through surface tension measurements. Brij 35, Corexit 0600, and Tween 40 all exhibited adsorptive tendency to Jordan sand and octadecane-coated Jordan sand. Triton X-114 adsorbed to octadecane-coated sand but not to clean sand. Surfactant adsorption to octadecane-coated Jordan sand was higher than adsorption to clean sand which represents a matrix with lower organic carbon fraction than octadecane-coated sand.

The oxygen uptake and protein production data from the biodegradation experiments was analyzed using a model that describes growth and oxygen utilization by bacterial cells metabolizing a soluble substrate in a suspending water phase with excess substrate present as a separate solid phase. BRKSO2 describes rates of dissolution of insoluble substrate, bacterial growth (with endogenous decay) on the primary substrate and/or a second substrate such as the soluble surfactant, substrate disappearance, and utilization of dissolved oxygen.

Model parameters that were used to obtain good simulations of the experimental data confirmed findings from the biodegradation experiments. Addition of Corexit 0600 and Tween 40 enhance octadecane biodegradation rates by increasing the dissolution rate of octadecane. Addition of Brij 35 has no significant effect on octadecane biodegradation and Triton X-114 appears to be inhibitory to octadecane biodegradation.

Model simulations predict that rate of biodegradation is limited by rate of dissolution of substrate. The calculated values of soluble substrate are shown to be very low throughout the batch test sequence.

The presence of octadecane as a coated film resulted in higher oxygen uptake rates which could be attributed to both higher octadecane dissolution rates and higher growth rates. It appears that enhancement of dissolution and growth rates due to surfactant addition (Corexit and Tween) was not as significant as enhancement due to the presence of coated octadecane.

The fact the data can be simulated well by the BRKSO2 model gives support to the conclusion that removal of separate phase octadecane is rate limited by dissolution. However, the possibility that direct octadecane transport to cells that colonize the surfaces of octadecane can not be excluded.

Complete report on this phase of the study is presented in Attachment B.

CONCLUSIONS

Conslusions are presented separately for each of the major chapters B.2 through B.5. Complete description of methods, results, and discussions are presented in Attachment B.

Solubilization of Octadecane in Surfactant Solutions

The presence of the four selected nonionic surfactants enhances dispersion of octadecane-surfactant complexes both below and above the surfactants' critical micelle concentration resulting in significant increase in apparent solubility of octadecane in surfactant solutions. However, the addition of the surfactants does not appear to enhance octadecane aqueous solubility at the molecular level. Total octadecane solubilization appears to be affected by addition of energy, with more octadecane dispersed in solutions that were shaken compared to unshaken samples.

Significant increase in octadecane dispersion due to the presence of surfactant suggests that removal and transport of octadecane by flushing would be greatly increased if surfactants are added. In addition, it is to be expected that dispersion would also result in an increase in the wetted surface area of octadecane thus increase potential solubilization rates and hence biodegradation rates. Surfactant adsorption, biodegradation, and possible toxicity to biomass are explored in the other chapters in order to form a more complete picture of surfactants' effect on bioremediation.

Adsorption of Surfactants on Jordan Sand and Octadecane-Coated Jordan Sand

The conclusion that some nonionic surfactants can adsorb to a soil, even a soil with extremely low organic carbon content, has several important implications. Adsorbed surfactants can increase the organic carbon content of a soil, thus increasing the soil's sorptive capacity. Surfactant sorption onto soil can also decrease the amount of surfactant available for desorption and solubilization of hydrophobic compounds. In addition, surfactant molecules adsorbed on soil can retain with them the hydrophobic compounds that they have solubilized and/or mobilized thus reversing surfactants' effectiveness in enhancing bioavailability of hydrophobic organics.

Thus, surfactant sorption should be a primary factor to be considered in the selection of surfactants to be used in contaminated soil cleanup and bioremediation. This is especially crucial if low (sub-CMC) concentrations of surfactants are to be applied to accommodate possible toxicity of high surfactant levels to microorganisms because the amount of surfactant available for solubilization may be reduced significantly due to surfactant adsorption.

So from the view point of adsorption, Triton X-114 would be the most desirable surfactant to be used in soil cleanup out of the four tested surfactants. Corexit 0600, then Tween 40 would be the next best for low foc soils, followed last by Brij 35. It is worthwhile to note, however, that these adsorption studies were done in batch systems where adequate contact time as well as mechanical agitation were provided and equilibrium was assumed to be achieved. In continuous flow systems, extent of adsorption may be different since contact time would be determined by flow rate and/or pumping rate. Also, contacting between surfactant and soil as well as surfactant with organics would be quite different, particularly as the energy input would affect the latter. Finally, the surfactants' abilities to solubilize and enhance biodegradation of hydrophobic substances should also be taken into consideration in the selection of surfactants for contaminated soil cleanup and bioremediation. These concerns are addressed in Chapters 2 and 4, respectively.

Biodegradation of Octadecane in the Presence of Four Nonionic Surfactants

In batch reactor tests in which octadecane (200 mg/L) was added as particulates, the presence of three (Brij 35, Corexit 0600, and Tween 40) of the four nonionic surfactants that were tested in this study all enhanced biodegradation rates slightly. Increased rates were most noticeable during the first 150 hours when particles of octadecane were largest. It appears that addition of surfactants enhanced dispersion of octadecane, thereby creating additional surface contact with the water phase resulting in higher rates of dissolution and accelerated biodegradation rates. The implication is that the rate of dissolution is the rate limiting factor.

Results from experiments in which octadecane was introduced as a coating on the soil grains thereby creating large surface area exposure to the water phase gave significantly higher rates of biodegradation with and without surfactants. These results support the conclusion that solubilization is a rate limiting factor.

Furthermore, quasi-linear growth was observed in all experiments, indicating that growth was most likely dependent on the rate at which soluble octadecane becomes available. It is possible that exponential growth which typifies systems with available excess substrate did occur in the very first few hours of the experiments when cell mass concentration was low, however, after more cell mass accumulated, exponential growth was not observed.

Two of the tested surfactants were biodegraded by the octadecane-acclimated mixed culture. Surfactant biodegradation may lead to limited surfactant availability as well as excessive oxygen and nutrient demand which can be costly. On the other hand, however, concurrent surfactant biodegradation would result in accumulation of active biomass which may lead to more efficient removal of target contaminants, and also, eventual surfactant degradation may be desirable if surfactants are added in-situ.

The biodegradation rates obtained from this phase of the study will be analyzed using a computer model that describes growth and oxygen utilization of a slightly soluble substrate in the presence of excess substrate. The model will attempt to correlate the effects of dissolution/solubilization of excess substrate on biodegradation rates.

Modeling

The model studies show that addition of Corexit 0600 and Tween 40 enhanced octadecane biodegradation rates by increasing the dissolution rate of octadecane, shown in BRKSO2 by a change in the product KL*KSA (the mass transfer coefficient and the surface area parameter). Addition of Brij 35, however, had no significant effect on octadecane biodegradation. Triton X-114 appears to be inhibitory to octadecane biodegradation, simulated in BRKSO2 through incorporation of an inhibition coefficient K21 by the presence of a second compound (the surfactant).

The presence of octadecane as a coated film resulted in higher oxygen uptake rates which could be attributed to both higher octadecane dissolution rates and higher growth rates. In comparing KL*KSA and $\mu 1$ obtained from experiments with surfactant and particulate octadecane versus experiments with coated octadecane, it appears that enhancement of dissolution and growth rates due to surfactant addition (Corexit and Tween) was not as significant as enhancement due to the presence of coated octadecane.

KL*KSA and $\mu 1$ were increased significantly for the data from the Brij 35 and Corexit 0600 experiments with octadecane coated sand. For Tween 40, because KL*KSA was already enhanced through surfactant addition (discussed above), the presence of octadecane as a coated film on sand did not further enhance solubilization rate. However, the growth rate coefficient $\mu 1$ was increased in the presence of sand for the case with 200 mg/L Tween. With Triton X-114, no significant enhancement in growth rate coefficient $\mu 1$ was observed between the tests without soil versus with soil. However, an increase in the value of KL*KSA was observed for 200 mg/L Triton in the presence of soil.

As discussed above, analysis of the batch reactor test data using the BRKSO2 program gave reasonably good simulations of the measured rates and cumulative mass changes of oxygen consumption and cell mass protein as a function of time in the absence and presence of surfactants. The model appears to be capable of simulating the dominant processes, e.g. solubilization of slightly soluble octadecane, biodegradation, cell mass-protein accumulation, and oxygen consumption. The model is also capable of simulating the effects of concurrent metabolism and utilization of surfactant for growth and energy including mutual inhibition effects. It is therefore deemed to be a useful tool for examining the effects of individual operating variables.

Section C

Biodegradation of Excess Phenanthrene in Soils in the Presence of Surfactants

Abstract

This phase of the research addresses the effect of low surfactant concentrations on the biodegradation of slightly soluble organic compounds in the presence and absence of soil. Biodegradation of phenanthrene in excess of its aqueous solubility by an acclimated mixed culture was studied in the presence of nonionic commercial surfactants. Nonionic surfactants were selected over the other types of surfactants because of their higher hydrocarbon solubilizing power, weaker adsorption to charged sites, less toxicity to bacteria and poor foaming properties. This class of surfactants is generally available as 100% active material free of electrolyte and is compatible with all other types of surfactants. The surfactants used in this study were: Triton X-114, Triton X-100, Triton X-405, Corexit 0600, Corexit 7665, Corexit 8600, Brij 35 and Tween 40. Surfactants were tested to measure their effectiveness for increasing solubility of phenanthrene, their effects on surface tension of the water phase, their adsorption on the soil matrix, their biodegradability, their effect on the adsorption of phenanthrene and their effect on the rates of biodegradation of phenanthrene by an acclimated mixed culture.

Solubility enhancement studies of phenanthrene by the surfactants indicated relatively small effects at sub-micellar surfactant concentrations, large enhancements are mainly a micellar phenomena. Batch biodegradation studies using a phenanthrene acclimated enrichment culture, in which phenanthrene was available as particulates and as a surface coating on sand were carried in closed BOD bottles in the Hach manometric system. Phenanthrene coated sand was designed to simulate soil contaminated with excess phenanthrene which remained after evaporation of lighter hydrocarbon solvents. Addition of surfactants at 25 mg/L enhanced biodegradation rates as measured by oxygen uptake, protein production and disappearance of phenanthrene. None of the surfactants were biodegraded alone by this culture during these experiments nor did they exhibit toxic effects on the culture. Oxygen uptake, removal of phenanthrene and protein production rates versus time showed a quasi-linear pattern as a function of time indicating that soluble substrate availability was limited. A dynamic model which couples dissolution and biodegradation processes could adequately represent the experimental batch data. Modeling studies suggest that biodegradation was accelerated because the dissolution rates of phenanthrene increased in presence of the surfactants.

Sorption studies on Jordan aquifer sand indicated that this low-carbon aquifer material adsorbs small amounts of phenanthrene as well as surfactants. Surfactant sorption on the sand can also alter the soils sorptive properties for other chemicals. Sorption isotherms could be represented by a linear model.

Continuous flow column studies with phenanthrene coated Jordan sand were carried out to simulate groundwater flow conditions. The tests show that low surfactant concentrations were marginally beneficial in washing phenanthrene from precoated sand. However mineralization of phenanthrene in these same columns was significantly enhanced in presence of the same surfactants indicating that the coupling between surfactant mobilization and biodegradation results in acceleration of the clean up process.

The study indicated that surfactant selection for in situ bioremediation of insoluble hydrocarbons will depend on a large number of factors with main emphasis on its hydrocarbon solubilizing power, low toxicity to bacteria and the environment and low sorptive properties. Although not tested in these studies ultimate biodegradability would also be desirable.

Complete report on this phase of the study is presented in Attachment C.

CONCLUSIONS

Conslusions are presented separately for each of the major chapters C.2 through C.9. Complete description of methods, results, and discussions are presented in Attachment C.

Solubility Enhancement of Phenanthrene in Water by Commercial Nonionic Surfactants

This study indicates that commercial nonionic surfactants can enhance the aqueous solubility of sparingly soluble hydrocarbons. The apparent aqueous solubility of phenanthrene increased linearly above the CMC of all the surfactants. The slope could be used to determine the molar solubilization ratio and the partitioning of the compound between the micelle and the aqueous phases which gives a quantitative measure of the effectiveness of the surfactant. The increase in solubilization by the different surfactants is related to their nonpolar content. This study indicates that surfactant concentrations both below and above the CMC have an impact on the water solubility of sparingly soluble organic pollutants and facilitate their transport in contaminated groundwaters. However, the effect below the CMC is too small to be measured precisely. It is therefore concluded that the comparison of the effectiveness of different surfactants below the CMC be evaluated by other criteria than solubilization namely, the relative effects on biodegradation and/or the relative sorptive properties of the soil matrix if that is deemed to be an important practical consideration.

Biodegradation of Phenanthrene in The Presence of Nonionic Surfactants

The results of this study show that commercial nonionic surfactants can be used to enhance biodegradation rates of phenanthrene, a slightly slightly PAH, using low surfactant concentrations (below their CMCs). This effect is attributed primarily to acceleration of the rate of dissolution of excess separate phase phenanthrene by promoting formation of more interfacial area. This mixed culture was capable of degrading phenanthrene without accumulation of significant amounts of intermediates. All the phenanthrene was converted to carbon dioxide and cell mass. Quasi linear growth is observed both in the presence and absence of surfactants indicating that growth is limited by availability of transportable soluble substrate. The rate of dissolution of phenanthrene is thus the rate limiting step.

Biodegradation of Phenanthrene in Soil in the Presence of Nonionic Surfactants

The batch reactor test data clearly show that addition of 25 mg/L of surfactant in the tests with dispersed and coated phenanthrene increased biodegradation rates. The presence of surfactants tends to stabilize suspensions of particulates thereby exposing more surfaces of solid phase phenanthrene for solubilization as reported by other investigators. Larger surface area facilitates mass transfer at the interface. The mixed culture was capable of degrading phenanthrene without accumulation of significant amounts of intermediates. Quasi linear growth is observed in all cases indicating growth is limited by availability of transportable soluble substrate. Soil dispersion in presence of the surfactants should be an important consideration in surfactant selection.

Modeling of Surfactant Enhanced Biodegradation of Phenanthrene

This study indicates that batch reactor data can be utilized to assess the relative rates of solubilization and biodegradation for substrates with low aqueous solubility. Analysis of the batch reactor test data using the BRKSO2 program gave reasonably good predictions of the measured rates and cumulative mass changes of oxygen consumption and cell mass as a function of time. The model can simulate the dynamics of solubilization, biodegradation, cell growth and oxygen consumption reasonably. The model results support that dissolution of slightly soluble organic compounds are enhanced in the presence of surfactants. This is evidence by the increase in the KL*Ksa parameter. Sensitivity analysis indicate that the substrate removal rate at high dissolution rates is governed by growth kinetics while at lower solubilization rates dissolution governs.

Sorption of Phenanthrene on Aquifer Material: Effect of Nonionic Surfactants at a Low Concentration

The adsorption of phenanthrene can be adequately described by the linear isotherm model over low aqueous concentrations. Low concentrations of nonionic surfactants may affect the mobility of non polar compounds. In the presence of the surfactants, phenanthrene tends to sorb not only on the organic fraction of the sand but also partitions into the hydrophobic interior of the surfactant. As the surfactant sorbs to the sand, it takes the bound phenanthrene along with it. This explains the enhanced sorption in presence of the surfactants. The surface tension measurements can provide sorption data for surfactant concentrations below their critical micelle concentration. The adsorption of all the six nonionic surfactants could be modeled by the linear isotherm. Sorption of nonionic surfactants on sand has important practical implications. Sorptive properties of surfactants will have a significant impact on remediation of contaminated low-carbon aquifers. Surfactants can increase the fractional organic carbon of the soil and change its sorptive properties. Their presence will also impact the fate and movement of hydrophobic organic compounds. Sorption of surfactants will also affect the retardation coefficient in contaminant transport models.

Laboratory Studies of Nonionic Surfactant-Enhanced Washing of Phenanthrene from Jordan Sand

This study indicates that the addition of surfactants at a low concentration may be slightly beneficial in cleanup of contaminated soils. The presence of surfactants does enhance the dispersion of phenanthrene/surfactant complexes even at a surfactant concentration below the critical micelle concentration. However actual success in the field will depend on the site conditions, geology and material composition. In practical applications the surfactant adsorptive losses would represent a significant material cost. Significant organic carbon content of the soil will also increase the loss of surfactants due to sorption. Higher flushing rates maybe beneficial to some extent.

Biodegradation of Phenanthrene in Soil in the Presence of Nonionic Surfactants: Column Experiments

This study indicates that the presence of low concentration of nonionic surfactants maybe beneficial in mineralization of low aqueous solubility compounds. The surfactants were applied

at sub-cmc concentrations. They did not inhibit mineralization nor were any toxic effects evident. The mixed acclimated culture was capable of degrading phenanthrene in the columns without any significant initial lag period. The biodegradation rates exceeded the rate of dissolution of phenanthrene. The results from this study indicate that low concentrations of surfactants may promote mineralization of low aqueous solubility compounds even if the solubility enhancement may not be significantly high. Additional work is necessary to assess which type of surfactant is best suited for enhancing mineralization and at what optimum dose it should be used.

Summary:

This research explores the use of commercial surfactants at a low concentration to enhance biodegradation of slightly soluble organic compounds by increasing solubilization. Nonionic surfactants were selected over the other types of surfactants because of their higher hydrocarbon solubilizing power, weaker adsorption to charged sites, less toxicity to bacteria and poor foaming properties. Surfactants were used at a concentration of 25 mg/L in all experiments which was below their critical micelle concentration. The study focussed on low surfactant concentrations because it seems unlikely that high concentrations of surfactants would be practical for insitu decontamination of soils by biodegradation both from the standpoint of toxicity to bacteria and potential oxygen demand created by ultimate biodegradation of the surfactant. Phenanthrene was used as a model compound because of its low aqueous solubility and high sorptive properties. It has also been identified as a major pollutant at numerous contaminated soil siltes. Biodegradation studies were carried out with phenanthrene available in the particulate form and as a surface coating on sand. Phenanthrene coated sand was designed to simulate soil contaminated with excess phenanthrene which remained after evaporation of lighter hydrocarbon solvents. The sand used in experiments was characterized by a low organic carbon content.

Conclusions:

(a) Solubility enhancement of phenanthrene by nonionic surfactants is mainly a micellar phenomena.

(b) The rate of biodegradation was enhanced in batch studies of phenanthrene by all surfactants. This was observed both when phenanthrene was available as particles and as a surface coating.

(c) The mixed culture was capable of degrading phenanthrene without accumulation of significant amounts of intermediates.

(d) Quasi linear nature of the oxygen uptake, protein production and phenanthrene removal curves indicate that rates of cell growth are limited by availability of soluble substrate.

(e) The numerical model BRKSO2 could adequately describe the oxygen uptake, cell growth and substrate disappearance data observed experimentally. Model studies supported that the most significant effect of surfactant addition was the iincrease in dissolution rates.

(f) Sorption of phenanthrene on sand could be represented by the linear isotherm model. The sorptive properties of phenanthrene was significantly enhanced in the presence of the surfactants.

(g) Column flushing studies with phenanthrene coated on sand indicated that the addition of low surfactant concentration was marginally beneficial.

(h) Biodegradation of phenanthrene was significantly enhanced in continuous flow column studies in the presence of some surfactants.

(i) Studies of cell transport in the soil column indicate that there maybe favorable retention of cells in soils which are contaminated by excess phase hydrocarbons.

(j) Surfactant selection for bioremediation of contaminated soils will depend on these major factors; hydrocarbon solubilizing power, low toxicity to bacteria and the environment, poor sorptive properties and ultimate biodegradability.